

STUDIES ON THE BLOOD VASCULAR TREE

I. A COMPOSITE STUDY OF THE FEMORAL ARTERY

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FIVE FIGURES

The constant presence of variations in the origin of the branches of the larger arterial trunks of the body is evident from even general observations in the laboratory during regular class dissections. Hitzrot's study of the axillary artery and Bean's on the subclavian artery disclose the fact, that the variations in the origin of the branches of these vessels conformed to well defined anatomic types. At the suggestion of Prof. J. Parsons Schaeffer a study of the blood vascular tree was begun at the beginning of the college year, 1914. In this the first paper, a study of the femoral artery will be presented. I wish to take this opportunity of acknowledging my thanks and gratitude to Doctor Schaeffer for the interest and attention he has given every detail of this study.

The records which underlie the present study were made from dissections by students and from personal dissections at the Daniel Baugh Institute of Anatomy of the Jefferson Medical College. Dissections of fifty-three cadavers were recorded: twenty-eight male white, six female white, four female negro, and fifteen male negro. There were forty-nine dissections from the left side of the body and fifty from the right side, ninety-nine in all.

While many minor variations not entirely in accord with the described types were observed in this study, yet the cases permit of a classification into distinct types. The classification of the femoral artery into its types is based on the origin and distribution of the profunda femoris artery and the medial and lateral

circumflex arteries. Section A of this paper describes the various types. Section B contains a description of the origin and distribution of the individual branches. Section C summarizes and discusses the results of the present study.

SECTION A. DESCRIPTION OF TYPES

TYPE I

This type (fig. 1) is present in 40 per cent of the cases classified—14 per cent on the left side of the body and 26 per cent on the right side. In this type the medial and lateral circumflex femoral arteries both take origin from the profunda femoris artery. Each branch usually has a separate origin.

The lateral circumflex artery in 40 per cent of this type, takes the form of a double vessel. This additional vessel courses parallel to the descending branch of the lateral femoral circumflex. The external pudendal arteries arise from the femoral as separate branches. The superficial epigastric and the superficial circumflex iliac take origin from the femoral as a common trunk. The highest geniculate artery arises from the femoral cephalic to the adductor opening, ventral to the adductor magnus muscle. There are in this type twenty-four male white, four female white, ten male negro, and two female negro subjects. This is the type described in the English, German and French anatomical text-books most universally used.

TYPE II

This type (fig. 2) occurs in 25 per cent of the cases classified—19 per cent on the left side of the body and 6 per cent on the right side. The medial circumflex takes origin as a separate branch from the trunk of the femoral artery. The lateral circumflex arises from the profunda femoris artery. The lateral circumflex in 20 per cent of this type is represented by an additional descending branch as in Type I. The superficial epigastric and superficial circumflex iliac arteries arise from the femoral as separate branches. The external pudendal arteries take origin

from the femoral in a common trunk. The highest geniculate artery arises in the same manner as Type I. There are of this

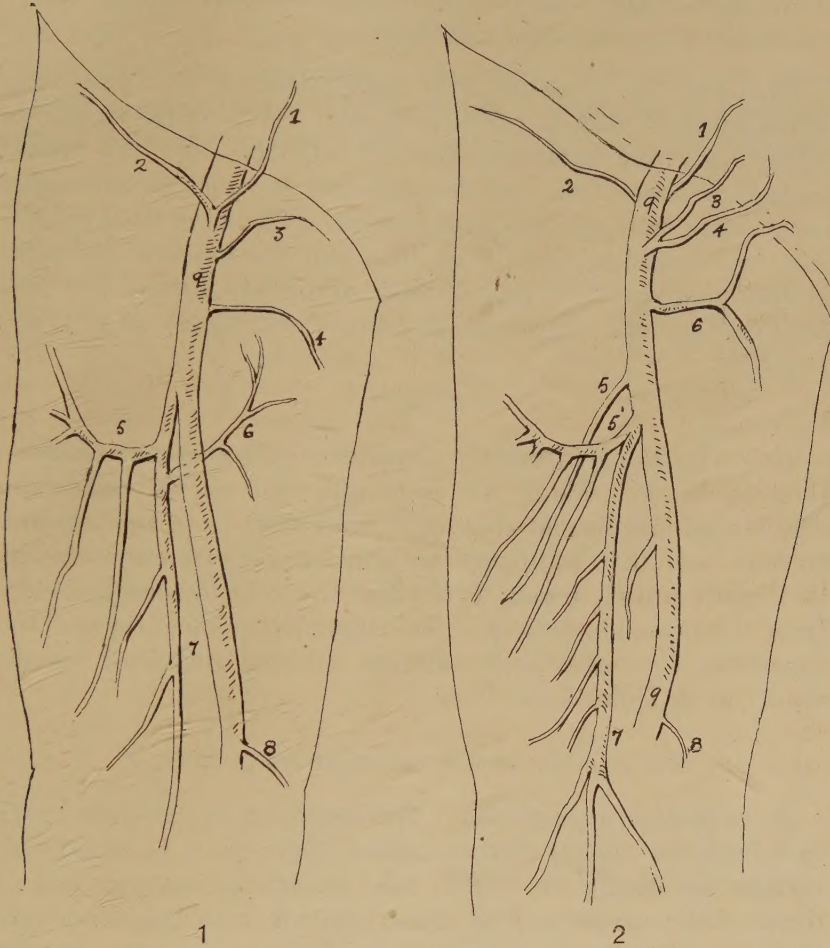


Fig. 1 This arrangement of the branches occurs in 40 per cent of the cases classified. The lettering is alike on all the figures and is as follows: 1, A. epigastrica superficialis; 2, A. circumflexa ilium superficialis; 3, A. pudenda externa superficialis; 4, A. pudenda externa profundus; 5, A. circumflexa femoris lateralis; 6, A. circumflexa femoris medialis; 7, A. profunda femoris; 8, A. genu suprema; 9, A. femoralis.

Fig. 2 This type is found in 25 per cent of the extremities observed. For index to lettering refer to figure 1.

type twenty-five subjects in all; twelve male white, two female white, ten male negro, and one female negro.

TYPE III

This type (fig. 3) occurs with slight variations in 19 per cent of the subjects studied, nineteen in all. Seven on the right side of the body and twelve on the left. In this type the lateral circumflex arises from the femoral and the medial circumflex takes origin from the profunda femoris. The superficial epigastric, the superficial circumflex iliac and the external pudendal arteries arise, in 52 per cent of these subjects in a common trunk from the femoral. The remaining 48 per cent ramify as in Types I and II.

TYPE IV

This type (fig. 4) is found in 11 per cent of the cases classified—three on the left side of the body and eight on the right side. The lateral and medial circumflex both arise from the femoral. In four cases there is present an additional lateral circumflex as in Types I and II, arising three times from the femoral and once from the profunda femoris. The superficial epigastric and the superficial circumflex iliac and the external pudendal vessels ramify as in Types I and II.

SECTION B. DESCRIPTION OF BRANCHES

A. epigastrica superficialis. This vessel arises in almost every case from the ventral aspect of the *A. femoralis*. In eighty-two extremities studied in which this branch is followed out, it occurs forty-one times in a trunk common with the *A. circumflexa superficialis*, twenty-nine times as a separate branch, and twelve times in a trunk common with the *A. pudenda externa superficialis* and the *A. circumflexa ilium superficialis*.

A. circumflexa ilium superficialis. This artery is worked out in eighty-two extremities. It occurs twenty-nine times as a separate branch, forty-one times in a trunk common with the *A. epigastrica superficialis*, and twelve times in a trunk common

with the A. epigastrica superficialis and the A. pudenda externa superficialis.

A. pudenda externa superficialis. This vessel arises in 28 per cent of the subjects studied in a common trunk with the A. pudenda externa profundus. This artery is absent nine times. It takes origin twelve times from the A. femoralis in a common trunk with the A. epigastrica superficialis and A. circumflexa

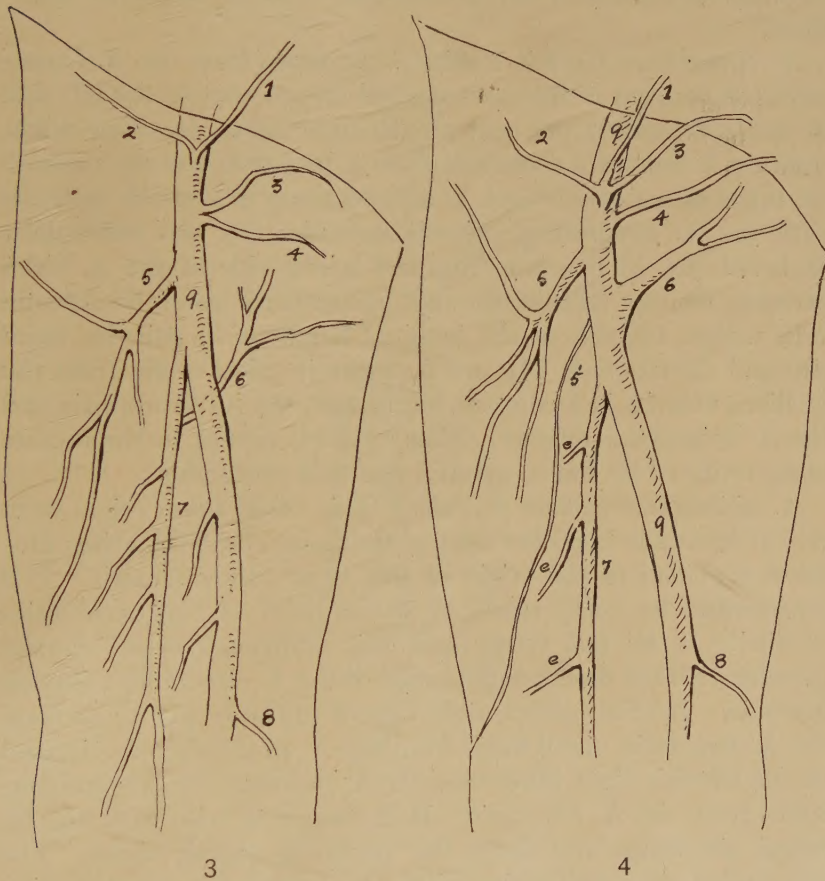


Fig. 3 This type occurs in 19 per cent of the vessels studied. For index to lettering see figure 1.

Fig. 4 This type is present in 11 per cent of the vessels observed. For index to lettering see figure 1.

ilium superficialis. Its most common origin is as a separate branch from the medial side of the A. femoralis.

A. pudenda externa profundus. It arises in 11 per cent of the extremities observed from the A. circumflexa femoris medialis. In 28 per cent it takes origin from the A. femoralis in a trunk common with the A. pudenda externa superficialis. In 52 per cent of the arteries observed, it takes origin as a single branch from the medial side of the A. femoralis. It is found absent seven times.

A. circumflexa femoris medialis. It arises from the A. femoralis in 36 per cent of the cases studied, 24 per cent on the left side of the body and 12 per cent on the right side. It takes origin from the A. femoralis cephalad of the A. profunda femoris, twenty-six times as a single branch and three times in a trunk common with the A. circumflexa femoris lateralis. In two extremities observed (fig. 5) it arises from the lateral side of the A. iliaca externa, courses distalward and medialward over the femoral vein within 1.5 cm. of the inguinal ligament to gain its usual site and distribution. In one instance it takes origin from the A. iliaca externa in a common trunk with the A. obturatoria and the A. epigastrica inferior. This vessel is absent in three cases being replaced by the A. externa pudenda profundus.

A. circumflexa femoris lateralis. This vessel takes origin from the A. femoralis in 30 per cent of the cases observed, arises nine times cephalad of the origin of the A. profunda femoris. It is represented by two vessels in 25 per cent of the extremities studied. In all but three cases this additional vessel courses parallel with the descending branch of the A. circumflexa femoris lateralis. It arises on the right side of the body five times from the A. femoralis, eight times from the A. profunda femoris; and on the left side eight times from the A. profunda femoris and four times from the A. femoralis. Both branches arise from the femoral six times and from the profunda eleven times. The descending rami outnumber both the transverse and ascending branches. In 8 per cent of the arteries classified, this vessel takes the form of an arterial arcade. It arises three times from the A. femoralis in a trunk common with the A. circumflexa

femoris medialis above the A. profunda femoris. It takes origin from the A. profunda femoris in 69 per cent of the cases classified.

A. profunda femoris. Its point of origin from the A. femoralis is variable. It arises four times at the level of the inguinal ligament (Poupart's ligament). It is distant from the inguinal ligament 1 to 2 cm. in 22 per cent of the extremities studied,

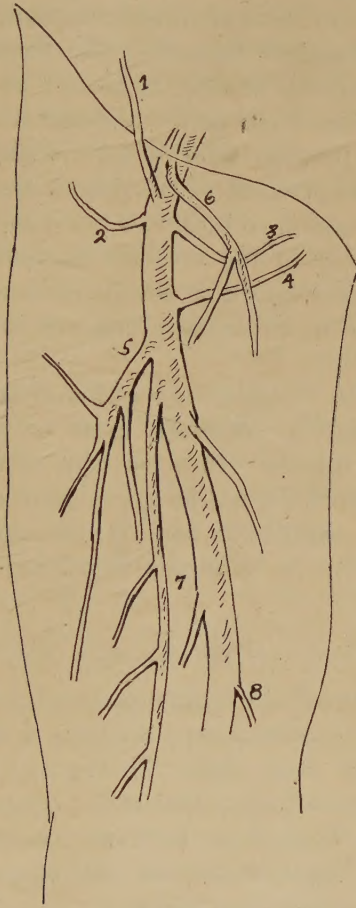


Fig. 5 This unusual arrangement, in which the A. circumflexa femoralis medialis takes origin from the A. iliaca externa and pursues a course medialward and caudad over the femoral vein and artery to gain its usual site is found in 2 per cent of the cases classified.

2 to 3 cm. distant in 49 per cent, 3 to 5 cm. distant in 18 per cent, and in one instance 9.5 cm. distant. In two extremities observed the A. profunda femoris arises from the lateral side of the A. femoralis and courses cephalad and medialward over the A. and V. femoralis to gain its usual site. There are two Aa. perforantes in 12 per cent of the arteries observed, three in 38 per cent, four in 41 per cent, and five in 9 per cent. The profunda femoris perforates the adductor magnus muscle at its termination as two or three terminal branches, less frequently as a single branch. The profunda femoris is absent as such in one case observed, i.e., it shows complete dissociation. The lateral and medial circumflex femoral arteries take origin from the A. profunda femoris in 40 per cent of the extremities observed. They arise in a trunk common to both in 9 per cent of the cases classified. In 36 per cent of the subjects studied, the A. profunda femoris does not give origin to the A. circumflexa medialis and fails to give off the A. circumflexa lateralis in 30 per cent of the cases observed.

A. genu suprema. This artery is found to take origin most frequently from the A. femoralis close to the opening in the adductor magnus muscle. In 8 per cent of the cases observed, it occurs as a branch from the upper part of the A. poplitea. Its branches, R. saphenous and R. musculo-articularis arise separately from the A. femoralis in 12 per cent of the cases classified.

SECTION C. SUMMARY AND DISCUSSION

1. A comparison of the branches of both sides of the body demonstrates the predominance of Type I (fig. 1) on the right side of the body and Type II (fig. 2) on the left side. Cunningham, Piersol, Quain, Testut and other standard anatomical treatises give Type I as the most frequent. A similar arrangement of the branches on each side of the body is present in 40 per cent of the subjects studied.

2. There are twenty-eight male white, six female white, three female negro, and sixteen male negro subjects included in this study. No relation of the branches to age could be drawn, as

there are only adults in this series. Thirty per cent of the dissections were made on negro subjects. The latter showed an unusually large number of variations and abnormalities. The negro subjects presented a greater proportionate number of variations and anomalies than the white.

3. The branches of the femoral artery differ in their origin on each side of the body in 60 per cent of the extremities studied. The representation of the lateral circumflex femoral artery as a twin vessel is of more frequent occurrence on the right side of the body. The medial circumflex femoral artery arises more frequently from the femoral artery on the left side of the body. The origin of the lateral circumflex artery from the femoral, either as a single or double vessel is found to occur with more frequency on the right side of the body.

4. Anomalies when present are not found as a rule on both sides of the body. The origin of the medial circumflex femoral artery from the external iliac taking the course medialward and caudad over the femoral vein to gain its usual site is found in 2 per cent of the cases observed. Another anomaly is found in connection with the medial circumflex, the latter arising from the external iliac in a trunk common with the obturator and inferior epigastric arteries. This arrangement is found in one subject and on the right side of the body. The origin of the lateral and medial circumflex arteries in a common trunk from the femoral is found in 3 per cent of the cases classified. The lateral circumflex is present as a twin vessel in 25 per cent of the cases observed. The profunda femoris artery is found absent in one case; in this instance, the lateral and medial circumflex in the femoral give origin to the perforating branches.

An anomaly not observed in this series is the presence of a double femoral artery. The femoral artery after giving off the profunda divides into two stems which reunite at a variable distance above the adductor opening so as to form a single popliteal artery. Quain reports one in twelve hundred bodies. Musgrove reviews double femoral arteries and found six reported in the literature. Bianchi and Chretien report cases in which this rare anomaly is found.

A very rare and interesting anomaly is the persistence of the *A. comes nervi ischiadici* as the principal vessel of the thigh—a condition which is found in all lower vertebrates. A. Manno reviews this anomaly. This unusual variation represents a cessation in the development of the femoral artery. Primarily the *A. glutea inferior* (sciatic artery) is the main arterial stem of the lower extremity, extending the entire length of the dorsal surface of the limb to the plantar surface, where it divides into the digital branches. The external iliac at this stage is small and terminates as the *profunda femoris*. In the lower vertebrates the inferior gluteal (sciatic) is the principal artery of the thigh and becomes continuous with the popliteal, the femoral being relatively insignificant, terminates as the *profunda femoris*.

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THREE CASES OF THE PERSISTENCE OF THE LEFT SUPERIOR VENA CAVA

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THREE FIGURES

Each of the cases of persistent left superior vena cava about to be described is of special interest from the fact that each presents an interesting and somewhat rare condition of the relations of the left superior vena cava to the other venous systems and to the heart. In the first case there is a persistence of the primitive venous system—the cardinal veins—without an apparent attempt at the metamorphosis to the adult condition. In the second case the left superior vena cava not only has the usual origin, course, and termination of this vein but also communicates by a large oval foramen with the left atrium. The left superior vena cava in case three terminates in the left superior pulmonary vein.

Upon reviewing the literature for the reported cases of double superior vena cava the writer has had the opportunity to consult the excellent papers on this subject by Marshall ('50), Gruber ('64), Bauer ('96) and Ancel and Villemain ('08) from whose publications he has freely drawn for certain cited cases, for which the original account was inaccessible.

The above mentioned authors have collected and reported 91 cases of persistent left superior vena cava. To this number the writer will add 29 older and more recent observations. For convenience they have been placed in tabulated form.

Summary of reported cases of double superior vena cava

	ADULTS	CHILDREN 1-11 YR.	NEWBORN	FETUSES	UNCLASSI- FIED	TOTAL
Double superior vena cava without anastomosis.....	17	5	10	19	13	
Double superior vena cava with small anastomosis...	10	2		2		
Double superior vena cava with normal anastomosis	9		3	4	2	
Left superior vena cava without right.....	6		1	3	2	
Persistent left superior vena cava unclassified....	2		2	2	6	
Total.....	44	7	16	30	23	120

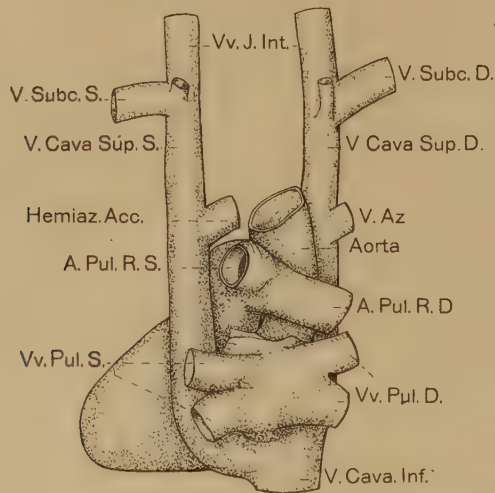


Fig. 1. The dorsal aspect of the heart and great vessels of an adult female showing the origin, course and tributaries of the right and the left superior venae cavae. One-third natural size.

Case 1 was a well nourished female subject of about 35 years of age. The clinical history was not obtainable. The heart shown in figure 1 was of normal size. The grooves on the surfaces were well marked. There were no defects in the interventricular or interatrial septa. The foramen ovale is completely closed. The systemic and pulmonary aortae take origin from the base of the heart and follow the normal course of these vessels. The right superior vena cava

is formed in the usual way by the union of the internal jugular and subclavian veins. It courses downward in front of the root of the right lung, to the right and posterior to the ascending aorta and terminates in the superior portion of the right atrium. At about 5 cm. below its formation the superior vena cava receives the azygos vein on its posterior wall. The left superior vena cava like the right is formed by the confluence of the internal jugular and subclavian veins and courses downward anterior and to the left of the aortic arch, anterior to the left pulmonary veins, then passes over the surface of the left atrium to enter the coronary sulcus. It courses to the right around the base of the heart in this groove and terminates in the right atrium anterior and to the left of the opening of the inferior vena cava. At about 5 cm. below its formation it receives the left azygos vein. There is no cross branch uniting the two superior venae cavae, which are of equal size.

By referring to the table it will be seen that there have been 64 cases of this type of duplication of the superior vena cava reported, only 17 of which could be definitely determined as having been observed in adults. Of the remainder 13 could not be classified with regard to the age of the subject.

Case 2 was a well developed male subject about 45 years old. The heart from this subject is very much enlarged. The atria are equal in volume to the ventricles. The left ventricle shares equally with the right in forming the anterior surface. The auricles are much elongated and have fimbriated margins. The aortae have the usual position and relations. The right superior vena cava is formed as usual and takes the ordinary course downward to the right atrium. At about 2.5 cm. below its formation it receives a cross branch (innominate) from the left superior vena cava. 7 cm. below its formation it receives the azygos vein.

The left superior vena cava originates from the union of the left internal jugular and subclavian veins. It passes downward for 2.5 cm. where it sends a branch to the right superior cava. Below this point it becomes reduced to about one-half its former diameter and courses downward anterior to the left pulmonary artery and left superior pulmonary vein and onto the surface of the left atrium. Here it becomes dilated to about four times its former diameter and crosses the surface of the left atrium to reach the atrio-ventricular groove. It courses in this groove to the right around the base of the heart and terminates in the right atrium anterior and to the left of the termination of the inferior vena cava. At the level of the inferior pulmonary vein in its course over the surface of the left atrium in the terminal dilated portion of the left superior vena cava is a large oval foramen, 3.5 cm. in a vertical diameter and 1.5 cm. in a horizontal diameter, which communicates with the left atrium.

Although the duplication of the superior vena cava has been observed to be relatively frequent in man the writer has been unable to find a reported case where the left superior vena cava communicated with the left atrium. However, of interest in this connection is the case of Ring (1805), in which the left superior vena cava terminated in the left atrium together with the inferior vena cava. Büttner-Weese (1769-1819) described one case and Breschet ('26) two cases where the left superior vena

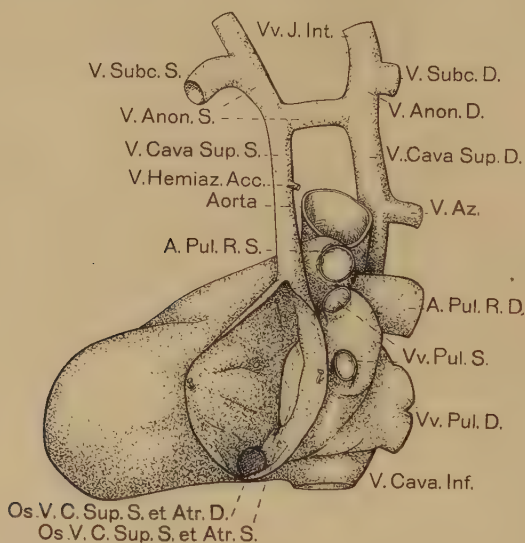


Fig. 2. The heart and great vessels of an adult male viewed from the left and dorsally. It shows the origin and course of the left superior vena cava and its communication with the right and the left atrium. One-third natural size.

cava terminated in the left portion of a common atrium in the newborn. Martin ('26) states that he saw, in a 1.5 months old child, the left superior cava ending in the left atrium. There were also many other disturbances of development. In Gruber's ('46) case the left superior vena cava terminated in the highest portion of the left atrium. Luschka ('62) described the left superior vena cava in a newborn child that terminated in the left atrium just anterior to entrance of the left pulmonary veins. It will be seen that the reported cases of left superior vena cava

have been observed only in very young infants and in the majority of them this anomaly was accompanied by other disturbances of development. It is questionable if Gruber's case should be included in this group because this anomaly might be produced by a union between the left superior pulmonary vein and the left superior vena cava, the union between the former and the lung having been lost early.

In this category may be included, also, those cases where the coronary sinus communicated with the left atrium but where the left primitive anterior veins have passed through the usual metamorphosis to the adult human condition. Four of these cases have been reported. Lindner (1787) observed an instance where the coronary sinus terminated in the left atrium. Jeffray describes the same condition in the heart of a fetus. Meckel (1816) reports a similar condition in his text-book. A case was seen by Bauer ('96) in which he describes very carefully a coronary sinus that communicated with the left atrium by an oval foramen 14 mm. and 11 mm. in transverse and antero-posterior diameter respectively. The usual communication between the coronary sinus and right atrium was completely closed.

Case 3 is a well developed male subject 57 years of age. The heart as shown in figure 3 is apparently normal. The aortae and the right superior vena cava have their usual relations. The left superior vena cava is formed by a very large subclavian vein uniting with a small internal jugular. It courses obliquely downward and to the right for 3 cm. where a large branch passes across the mid-line to the right superior cava. Below the point where the branch is given off to the right side the left superior vena cava is about one-third its original diameter. After coursing downwards for 4 cm. it terminates in the left superior pulmonary vein. The pulmonary vein, which appears to be situated higher in the hilum than usual, is formed by the confluence of three veins from the upper lobe of the left lung. After coursing downward and to the right it terminates in the upper part of the posterior wall of the left atrium. The left inferior pulmonary vein has the usual course and termination.

The somewhat rare condition of a communication between the left pulmonary veins and the persistent left superior vena cava or with a persisting portion of this channel has been reported eleven times. These cases may be separated into four groups

according to the portion of the anterior cardinal vein that remains patent and offers a drainage channel for a portion of the left lung either into the right atrium by way of the innominate vein or the coronary sinus or as a channel by which a large part of the venous blood from the upper part of the left side of the body reaches the left atrium.

In those cases where the left superior vena cava has the usual origin, course and termination, Wilson (1798) was the first to report a case where left pulmonary veins terminated in the

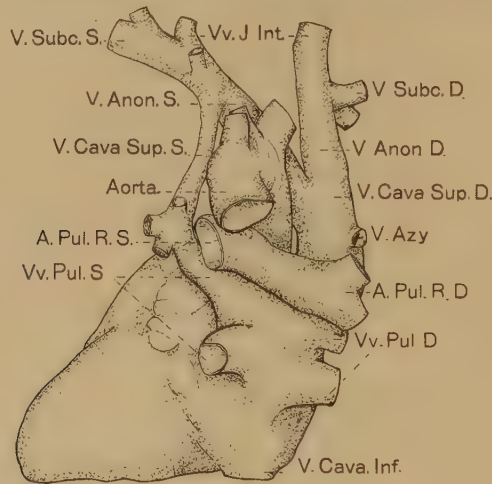


Fig. 3. The heart and great vessels of an adult male viewed from the left and dorsally. It shows the origin and course of the left superior vena cava and its termination in the left superior pulmonary vein. One-third natural size.

left superior vena cava. His case was a malformed child. Therman ('95) described a similar condition in a child two months old. In the second class of cases the left superior vena cava has the usual origin of this vein; it courses downward and terminates in the pulmonary vein which terminates as usual. In these cases there is a well formed coronary sinus. Case 3 as described in this communication is of this type. Hyrtl ('39) observed 2 cases, one in a newborn female child the other in a 60-year-old male, where the left superior vena cava joined the left superior pulmonary vein which terminated as usual in the

left atrium. Somewhat more recently Revilliod ('89) described a condition where the upper persisting portion of the anterior cardinal veins entered the left atrium together with the left pulmonary veins. This anomaly occurred in a 3 months old female child.

The third group of cases presents the proximal portion of the anterior cardinal veins alone persisting and gives the appearance of the coronary sinus originating in the pulmonary veins. The pulmonary veins have lost the usual connection with the left atrium. Hickman ('69) states that in a female 28 years of age the pulmonary veins from the left lung enter the right atrium. This interesting condition is produced by the union of the pulmonary veins with the coronary sinus through a persistent portion of the duct of Cuvier. Somewhat more clearly Nabarro ('03) describes this condition in a 5½ months old child. In this case all of the pulmonary veins opened into the right atrium by way of a large coronary sinus.

Finally, in the fourth group the persistent portion of the left anterior cardinal has lost its connection with the left duct of Cuvier (coronary sinus) which gives the appearance of the pulmonary veins terminating in the left innominate vein. The pulmonary veins in these cases have lost the connection with the left atrium. Blair ('02) claims that a pulmonary vein, normal in size, originating in the superior lobe of the left lung terminates in the left innominate vein. Somewhat similar was the case of Thane ('06) where a superior pulmonary vein entered the left innominate in an adult. Patterson ('13) discovered a case where the upper lobe and the superior half of the inferior lobe drained into the left innominate vein. Nützel ('14) states that Ramsbotham observed a case where the left superior pulmonary vein terminated in the left subclavian vein. Johnston ('15) very clearly describes a case where the left superior pulmonary vein joins the upper persisting portion of the left superior vena cava, the left superior vena cava terminated in the left innominate vein and the coronary sinus was completely formed.

Under this general grouping may also be included those cases reported where there is a communication between the right

superior pulmonary veins and the normal superior vena cava. The causative factors are the same in either case. Meckel ('20) observed the right superior pulmonary vein terminating in the superior cava in an adult. In his short notes Otto ('30) shows the pulmonary vein ending in the right atrium. Cooper ('36) and Chassinat ('36) each describe a case where the right pulmonary vein ended in the superior cava. Somewhat more clearly Lambl ('60) describes this abnormality. He reports the communication of the right pulmonary vein with the right atrium. Törster ('61) presents in his work on the anomalies of the human body a note on the origin or the termination of the pulmonary veins in the superior or the inferior vena cava without giving the details in these cases. Somewhat indefinitely Duchek ('62) states that he observed the left superior vena cava arising in the right superior pulmonary vein, in a male adult. Gruber ('76) states that he saw the right superior pulmonary vein communicating with the superior vena cava. Gegenbauer ('60) describes a case in a 2 year old child where the right superior pulmonary vein ends in the superior vena cava. Chiari ('80) observed the abnormal termination of the right pulmonary vein in the right atrium. Gruber ('80) described a case where the right pulmonary vein terminated in the superior vena cava in an adult female. Töpley ('82) observed this interesting abnormality: in a 20-year old male subject the superior vena cava is formed as usual; shortly after receiving the azygos vein it divides into two limbs, the ventral terminating as the ordinary vena cava superior and the posterior limb, after passing between the branches of the right pulmonary artery and bronchus, enters the left atrium. In a case having anomalies of many organs Epstein ('86) described the right and left pulmonary veins uniting to form a common trunk which courses behind the heart and terminates in the superior vena cava. Rokitansky ('86) mentions in his text-book on pathological anatomy a case of abnormal termination of the right pulmonary vein in the right superior cava. He also describes a case where the right pulmonary veins terminated in the right atrium. In his description of a double superior vena cava Hepburn ('87) has shown

that the right pulmonary vein opened into the right atrium. Miura ('89) describes the finding at autopsy on a 6 months old child the two right and the two left pulmonary veins united into a small vein on each side and these finally uniting to form a single trunk before terminating in the azygos vein. The azygos vein communicates with the upper part of the right atrium. Shepherd ('90) observed a case where the single right pulmonary vein terminated in the azygos vein just before it arched over the root of the lung. Audry and Lacroix ('90) mention a case where the right pulmonary veins joined the right atrial wall. Etlinger ('91) briefly states that he observed a similar case where the right pulmonary vein entered the right atrium. Ingalls ('07) states that two right pulmonary veins communicated with the superior cava just before it terminated in the right atrium in his case. A third pulmonary vein terminated as usual. Stoeber ('08) reported a case of 'cor triatriatum' where the pulmonary veins from the right and left lower lobes of the lungs terminated in the left atrium. The pulmonary veins from the upper lobes terminated in the right atrium. Schröder ('11) described a case which he observed in a 26-day old child where the left superior vena cava terminates in the left atrium and the right pulmonary veins communicate with the right innominate vein. Merkel ('12) has observed a case in a 70-year old male subject in which the right pulmonary vein communicated with the superior vena cava and also with the left atrium. Brown ('13) in describing Doctor Park's case states that the right pulmonary veins joined the superior cava. There were no right pulmonary veins that entered the left atrium. Gerard ('14) interestingly describes a case where the right superior pulmonary vein entered the superior cava. Nützel ('14) also describes a similar condition observed in a 47-year old subject.

The embryological significance of the persistence of the left superior vena cava as shown in figure 1 has been so frequently described that a detailed discussion of this unusual condition is deemed unnecessary at this time. Case 2, however, in which in addition to the complete persistence of the left anterior car-

dinal vein and duct of Cuvier there is a communication between this venous channel and the left atrium deserves further consideration. In the text-book of human embryology edited by Keibel and Mall it is stated that there develops in the early fetal heart, beside the atrial and ventricular chambers, a third chamber, known as the sinus venosus. The sinus venosus becomes elongated laterally into the right and left sinus horns. The sinus communicates with the common atrial chamber by a large oval foramen with its long axis placed transversely. During the normal course of development the left sinus horn becomes reduced in size, and finally loses its connection with the left cardinal vein. At about the same time the left half of the large oval foramen connecting these two chambers becomes constricted so that the opening becomes reduced to less than half its original diameter. The interatrial septum develops just to the left of this constricted opening. It appears quite probable that in Case 2 the left sinus horn and the oval foramen uniting the sinus venosus and the common atrial chamber did not undergo the usual changes as very briefly described above but developed proportionately with these structures on the right side. With the development of the interatrial septum, dividing the common atrium into right and left portions, the large oval foramen uniting the sinus venosus and common atrium, and which remained undiminished in size, also became divided into right and left portions and maintained a communication with the right and left atrial chambers respectively.

In his description of the development of the pulmonary veins for the cat Brown ('13) has shown in 4.5 mm. embryos and in his figure 1 that the anlage of the pulmonary veins and the anterior cardinal veins are connected with an indifferent capillary plexus surrounding the foregut and lung anlage. Undoubtedly, then, the communication of the pulmonary veins with the left superior vena cava as is shown in figure 3 is the persistence of the primary condition described above. In the writer's case that portion of the left anterior cardinal vein between the level of the superior pulmonary vein and the heart has undergone the usual changes that are found in man. The

left superior vena cava in this case gives the appearance of terminating directly in the superior pulmonary vein.

In conclusion I beg to thank Mr. Atwell for the execution of the drawings which were prepared according to the methods he described in volume 10 of the Anatomical Record.

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THE IMPLANTATION AND EARLY SEGMENTATION OF THE OVUM OF DIDELPHIS VIRGINIANA

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FIFTEEN FIGURES

Since few investigators have described the development of the opossum and since there is rather incomplete information at hand concerning the early stages of its development, it seems desirable that the subject be re-investigated. The following results will serve as a preliminary account of a more extensive work which will appear later. During the last five years we have collected and examined about fifty female opossums for embryos. We were unable to find any early stages of development until March 10, 1914.

November 2, 1913, six opossums, three males and three females were collected at Graydon Springs, Missouri. They were taken to Springfield, Missouri, and kept in a cage, made of chicken-wire netting, in which a large box was placed which served as a bed and hiding place. During February, 1914, the females were examined from time to time to see if the external genitals and marsupium, including the mammary glands, showed signs of oestrus such as congestion and enlargement of the external genitals and mammary glands and the moistening of the marsupium.

These conditions were observed February 12, 1914, in one large, adult female. This specimen was killed and the ovaries and the uteri were examined. The uteri were not enlarged and ovulation had not taken place as sections made later of the ovaries showed. On February 23, 1914 another large female was killed and examined when the above mentioned conditions

were observed and the uteri were not enlarged and ovulation had not taken place.

On March 10, 1914 a young female opossum was killed and examined about four days after the beginning of the oestral period. The remainder of this paper will deal principally with the material furnished by this specimen. The uteri were found enlarged at least six times the size of those of the two preceding specimens. Ovulation had taken place as the ruptured Graafian vesicles were visible to the unaided eye. The enlarged uteri were very soft, delicate and much congested. When an incision was made into the cavity of the uteri it was difficult to distinguish the cut surfaces of the uterine walls from their mucous lining. The walls of the uteri of this specimen are made up of a very delicate, homogeneous appearing, loosely constructed tissue which when sectioned was found to be almost entirely glandular as is shown in figures 12, 13 and 14.

The left uterus was cut open first and the walls were laid back with considerable difficulty. One might well have expected to find the large and distended uterus in an advanced stage of pregnancy but a careful examination of it revealed merely the presence of many, small, glistening, globular bodies scattered irregularly over the uterine mucosa in depressions. Osborn ('87) states that, "the inner wall of the uterus at the mid-period (of gestation) presented one or two long parallel furrows faintly defined upon its ventral surface, in which the embryos were ranged in a single row." At first these globular bodies which afterwards were found to be segmenting ova were scarcely visible to the unaided eye, due to the facts that they were more or less embedded in the uterine mucosa, that the mucosa was much congested and that they were more or less stained with blood. The left uterus with its segmenting ova intact was placed in toto in Alexander Petrunkevitch's sublimate mixture¹ for a few minutes. The globular bodies or segmenting ova rolled out of their cups or depressions in the uterine mucosa on slight

¹ The sublimate mixture of Alexander Petrunkevitch is made as follows: 40 per cent alcohol, 500 cc.; fuming nitric acid, 10 cc.; glacial acetic acid, 90 cc.; bichloride of mercury to make a saturate solution (about 10 grams).

disturbance after they had been placed in the killer. From the action of the killer the delicate ova suffered more contraction than did the uterine lining. This shrinking tended to separate the ova from their uterine cups.

A longitudinal incision was made the full length of the right uterus. When the walls were laid back many segmenting ova were found more or less embedded in the uterine mucosa as in the left uterus. Blocks of uterine tissue containing the ova were cut out with a razor and placed in the killer. Twenty-five ova were recovered from both uteri. We were able to carry twenty-two of the twenty-five ova through the histological process. The tissue was embedded in paraffin and cut into sections eight micra in thickness. It was stained in Heidenhain's iron-hematoxylin and hematoxylin and eosin. The iron-hematoxylin gave the better results.

Some of the tissue was sectioned through the ova perpendicular and some horizontal to the surface of the uterine mucosa. The perpendicular sections show that the ova are embedded about one-half their diameter and sometimes more deeply in cups in the uterine mucosa. According to Selenka ('85), the blastodermic vesicles lie at first quite free and at random in the uterus; on the fourth day (after the beginning of segmentation) the blastodermic vesicle over the germinal area becomes loosely adherent to the uterine epithelium. We find that the segmenting ova and even a one-celled ovum are embedded from the first in cups in the uterine mucosa as is well illustrated in figures 12, 13, 14 and 15. The horizontal sections show that the ova are in cups and not in straight grooves as Osborn ('87) found the embryos. The uterine mucosa is composed of three or four layers of cells but it is much flattened out where it comes in contact with the ova. The ova still have many follicular cells adhering to them.

These segmenting ova with their envelopes vary in diameter in the fresh state from about 0.75 to 1.50 mm. The ovum proper makes up relatively a small amount of the complete egg as can be seen in figures 12 and 14. The cytoplasm of the ovum is coarse, granular and much vacuolated. The egg envelopes are divided into two more or less distinct areas. The central one

which immediately surrounds the egg is composed of many concentric, coarse, reticular layers (fig. 11). The peripheral area is finely reticular and the layers are not well defined. The egg envelopes seem to be a coagulated white in the sections. The egg envelopes are so loosely constructed that the segmenting ova in some of the specimens have subsided and thus become eccentric in the envelopes during the histological process as can be seen in figures 13 and 14.

Selenka ('85) stated that the ovum of the opossum is intermediate in type between the meroblastic and holoblastic egg. It is difficult to understand the significance of this statement unless it refers to the yolk extrusions and the egg envelopes which do not segment. There are extrusions of yolk given off before the egg begins to segment. The egg proper is holoblastic. What we call the egg envelopes probably correspond to the accessory envelopes of the hen's egg.

Before or during the process of segmentation the ova have given off fragments or extrusions of cytoplasm or yolk. These

Fig. 1 A section of an ovum in the process of fertilization showing the fusion of the male and female pronuclei. The lower pronucleus which is probably male is more deeply stained than the upper female pronucleus. Correlated with the relatively numerous yolk extrusions is the small size of the ovum.

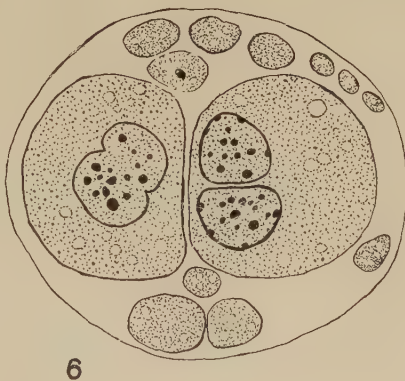
Fig. 2 An ovum in which the nucleus has divided into two nuclei but the cytoplasm shows no signs of division. There are relatively few yolk extrusions and the ovum is large.

Fig. 3 A two-celled stage of segmentation. The elongated nuclei indicate that the next division will be an equatorial one. The blastomeres are relatively small due to the large number of yolk extrusions.

Fig. 4 A two-celled stage of segmentation with the polar bodies. There are few yolk extrusions and the blastomeres are relatively large. Each blastomere has two oval nuclei; one of them is generally seen as a peculiar round body with large chromatin granules around its periphery. This may be the maternal derivative. It has been found that the pronuclei may segment before they fuse. These nuclear-like structures may be divided and unfused pronuclei. Professor E. G. Conklin has called such structures 'gonomeres.' These conditions resemble those in figure 1. It is very difficult sometimes to determine whether nuclei are dividing or fusing.

Fig. 5 A two-cell stage of segmentation with the polar bodies. There are very few yolk extrusions and the blastomeres are very large.

Fig. 6 A two-cell stage of segmentation in which the nucleus of one of the blastomeres has divided and in the other it has begun to divide. One polar body is noticeable.



fragments vary in size and number. Some of the ova have four or five fragments while others have as many as a dozen or more. Such extrusions lie around the blastomeres and are not scattered throughout the reticular egg envelopes (figs. 2, 4, 6 and 9). The ova and the blastomeres are smaller when they are surrounded by a large number of these fragments; conversely the blastomeres are larger when the fragments are fewer in number as can be seen in figures 1 and 3. The ova in the advanced stages of segmentation have fewer of these fragments than the one and two-celled stages. They have probably been reabsorbed as food. These extrusions do not contain nuclear or chromatic material; at least they do not take a nuclear stain. It is probable that these extrusions represent a normal condition. Judging from their constancy they would seem to be of significance. The fact that they resemble the cytoplasmic material would indicate that they had once been a portion of that material. According to Selenka ('87) these fragments of cytoplasm are enveloped by the blastomeres of the blastula and thus lie in the segmentation cavity.

The ovum of the opossum has marked polarity which is noticeable at the beginning of segmentation. The first and second polar bodies are sometimes evident in these ova. The egg envelopes which are probably nutritive do not segment. The first segmentation plane which is meridional divides the egg into two equal blastomeres as is shown in figures 3, 4 and 5. The second plane of division which is an equatorial one divides each of the first two blastomeres into two unequal cells; the smaller ones being at the animal pole. The second divisions are not simultaneous as is well shown in figures 6, 8 and 9. The division of the nucleus precedes by a long time that of the cytoplasm and of the cell wall as is illustrated by figures 2, 7 and 8. The third plane of division is meridional and divides the first four blastomeres into two cells each, thus giving the eight-celled stage which is shown in figure 10. The blastomeres of the early stages of segmentation do not adhere closely together.

The ova of this last described specimen varied in their development from one to eight cells. Two of the ova are in the one-



Fig. 7 A two-cell stage of segmentation in which the nuclei of both blastomeres have divided.

Fig. 8 A two-cell stage of segmentation with the polar bodies. The nuclei of both blastomeres have divided. The nuclei of the right blastomere have moved to opposite ends of the cell preparatory to the division of the cytoplasm. It will be noted in figures 6, 7 and 8 that the second plane of division is an equatorial one.

Fig. 9 A three-celled stage of segmentation in which the nucleus of the left blastomere is almost divided preparatory to the formation of the four-celled stage. Note the unequal distribution of the cytoplasm in the resultant right blastomeres.

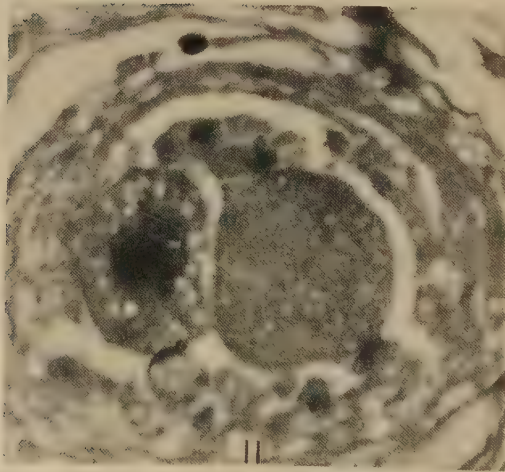


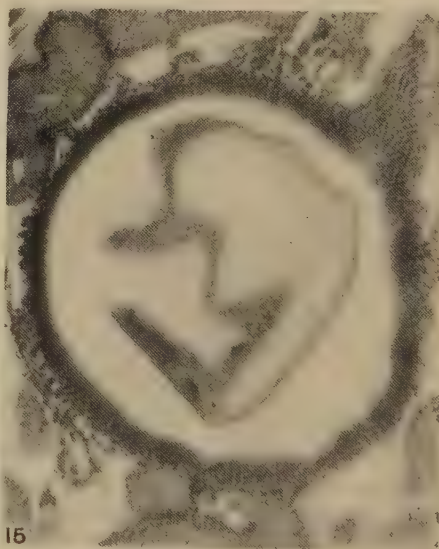
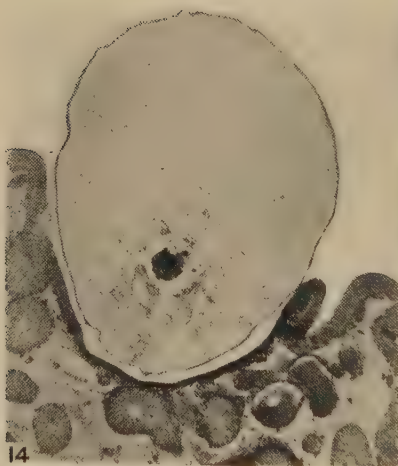
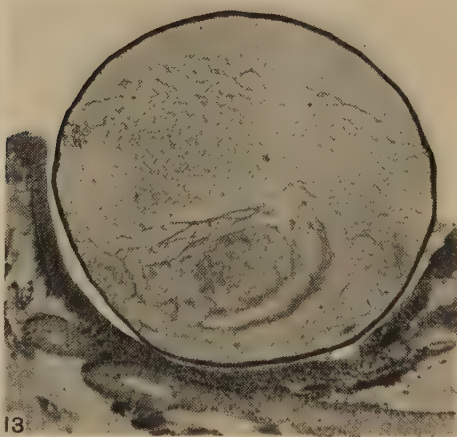
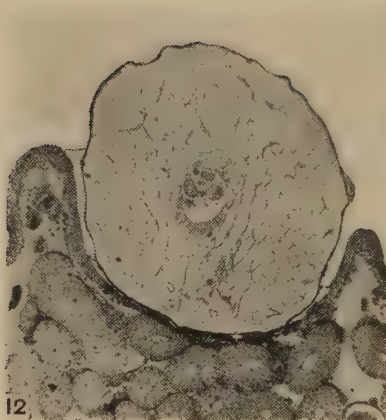
Fig. 10 An eight-cell stage of segmentation. The four blastomeres at the animal pole are smaller than those at the vegetative pole.

Fig. 11 Photomicrograph through the center of an egg to show the appearance of the central part of the egg envelopes. The egg is in the two-cell stage of segmentation with the polar bodies and many yolk extrusions.

cell stage, five in the two-cell stage, six in the two-cell stage with four nuclei, three in the eight-cell stage, two probably in the blastula stage and the other four are in the four-cell stage of segmentation.

Selenka ('85) found from nine to twenty-seven ova in the uteri. There are never as many embryos in the marsupium as there are ova in the uteri. Selenka never observed more than six embryos in the marsupium. We found one specimen with eight embryos in the marsupium. Most opossums, however, bring to maturity a smaller number owing to the fact that there is always a high rate of mortality for the developing ova. The difference in the number of the developing ova in the uteri and the number of the embryos in the marsupium is probably due in part to the difficulties encountered in the transfer because they are transferred at a very immature stage of development. According to Selenka gestation lasts exactly eight days. Taking into consideration the variation in the rate of segmentation of the ova of this opossum there would be considerable difference in the stages of maturity of the embryos at the end of eight days if the weaker ones did not die or become abnormal.

The sections from which the photomicrographs are taken are magnified about one hundred times. The drawings were made with the aid of a camera lucida and are magnified about six hundred times. The heavy line surrounding the ova in the drawings is not as uniform and continuous as it is illustrated. It is more of a compressed net than it is a continuous line. It appears to be a product of coagulation. It is not the zona which is a distinct membrane in the ova of most mammals. The drawings cover only the egg proper. The cytoplasm is more vacuolated than is shown in the drawings. If the egg envelopes were included in the drawings and drawn to the same scale the drawings would have an additional radius of from four to five inches. Figures 1 to 10 are drawings from sections. Figures 11 to 15 are photomicrographs.



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Figs. 12, 13 and 14 Photomicrographs from sections through or near the center of the egg, perpendicular to the uterine mucosa which show the implantation of the eggs in the uterine cups. The cups are cut in cross section. The cups are formed by circular folds of the uterine mucosa which extend well above its surface. Figures 12 and 14 illustrate very well the gross structure of the ovum. On the outside is seen the thick limiting membrane. Beneath this is a thick layer of a coagulated albuminous material which is limited internally by a sharply staining surface which is probably a coagulation product. This has been arbitrarily indicated by a solid line enclosing the ova in figures 1 to 10. Inside this demarcation is a less dense substance in which the blastomeres are suspended.

Fig. 15 Photomicrograph of a section through a uterine cup, horizontal to the uterine mucosa. The section is a little below the center of the egg.

BOOKS RECEIVED

The receipt of publications that may be sent to any of the five biological journals published by The Wistar Institute will be acknowledged under this heading. Short reviews of books that are of special interest to a large number of biologists will be published in this journal from time to time.

THE ALLIGATOR AND ITS ALLIES. Albert M. Reese, Ph.D., Professor of Zoölogy in West Virginia University (Author of "An Introduction to Vertebrate Embryology"), with 62 figures and 28 plates, 1915, \$2.50. New York: G. P. Putnam's Sons.

From the Preface: The purpose of this volume is to bring together in convenient form for the use of students of zoölogy some of the more important details of the biology, anatomy and development of the Crocodilia. For obvious reasons the American Alligator is the species chiefly used.

In the first chapter the discussion of the alligator is largely the result of the personal observations of the author; the facts in regard to the less familiar forms are taken from Ditmars and others. The description of the skeleton, with the exception of short quotations from Reynolds, is the author's.

The chapter on the muscular system is a translation from Bronn's *Thierreich*, and the author has not verified the descriptions of that writer.

The description of the nervous system is partly the author's and partly taken from Bronn and others.

The chapters on the digestive, urogenital, respiratory, and vascular systems are practically all from descriptions by the author.

The chapter on "The Development of the Alligator" is a reprint, with slight alterations, of the paper of that title published for the author by the Smithsonian Institution.

The bibliography, while not complete, will be found to contain most of the important works dealing with this group of reptiles.

ORIGIN OF THE BLOOD CELLS. DEVELOPMENT OF THE HAEMATOPOETIC ORGANS AND REGENERATION OF THE BLOOD CELLS FROM THE STAND-POINT OF THE MONOPHYLETIC SCHOOL¹

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ONE PLATE 4^{*}

The last century was marked by very intensive work in the field of haematology, including, as it does, erythropoiesis, lympho- and leucopoiesis. Many investigators have endeavored to throw light on such obscure problems as the embryonic and phylogenetic development of the blood, the structure of the haematopoietic organs, and the development of the vessels in which the differentiated products are conveyed into body tissues. Finally, the function of the different white blood cells has formed a new and interesting problem.

International coöperation has contributed certain results to this field of research, a part of which will have permanent value and form the basis for further investigations. I do not need to emphasize the merits of the investigators, to whom we owe an elucidation of the development of the lymphatics. Most of the students of blood embryogeny and blood phylogeny made their investigations in Europe. From what I can judge, America presents good opportunities for a rational understanding of the function of the lymphatic tissue, as is exhibited in some of the work now being done at the Rockefeller Institute.

The student of biology is engaged in a study of the development and function of living matter, but function is inseparably connected with structure. A correct understanding of the function of the different blood cells could not even be approached

¹ Lecture delivered before the College of Physicians and Surgeons, Columbia University, New York, on November 24, 1915.

some ten years ago. At that time the mutual relationship of the different blood cells was not established and there existed very few indications concerning the structure and localization of the haematopoietic organs. It is my intention to present here the results of those investigations which are principally concerned with the development and structure of the haematopoietic organs and of the elements which are differentiated in these organs.

A brief résumé of the knowledge of haematopoiesis during the last century may be permitted. The zoologists, embryologists and histologists considered the blood islands of the area opaca as the source from whence arose the first red blood cells. The concurrence of views disappeared, however, as soon as the question of the origin of the blood islands was considered.

Very definite indications have shown that the blood development in mammals is transferred in early embryonic life from the area vasculosa to the liver and finally to the bone marrow; but the search of a haematopoietic organ, homologous to the mammalian liver, was fruitless in the meroblastic eggs of the birds and reptiles.

The problem of the genesis of a new haematopoietic organ was readily answered. Blood cells, capable of mitosis, were ejected at certain times from the existing and functioning organ, as for instance, from the area vasculosa into the liver. Here they found favorable conditions for their development, multiplication and differentiation, and the new haematopoietic organ began to function. How radically this interpretation has changed will be shown in this communication.

The facts, reported above, concerned chiefly the erythropoiesis. The localization, the time and the source of the development of the white blood corpuscles were sometimes accounted for in a very peculiar way. The marked differences in the structure of the red and white blood corpuscles led to the assertion that these different cells must have a separate origin. The fact that white blood cells were not found in the vessels of young embryos and the presumably short duration of the haematopoietic function of the area vasculosa led many investigators to look for a development of leucocytes within the embryo body. Of course a separate

genesis of the red and white blood cells under such circumstances was taken for granted. The presumption that the erythrocytes developed exclusively in the blood islands and that the leucocytes differentiated later in the embryo body itself seemed to be unshakable. The observations of V. der Stricht, describing the presence of leucocytes in the area vasculosa of the chick embryo with two segments, stood isolated. The opinion of Saxer and Bryce recognizing a common stem-cell for the white and the red blood corpuscles, seemed to be in contradiction to all known facts.

The conception of the lymphatic cells of the thymus, as deriving from the epithelium, shows how paradoxical was the judgment about certain forms of the white blood corpuscles. The same origin was conceived for the spleen cells in the reptiles. This latter assumption again prompted the admission of different stem-cells in the case of the lymphocytes and the polymorphonuclear leucocytes.

In speaking of definite stem-cells I have in mind such kinds of cells generally, as may be differentiated and identified morphologically. Up to the present, a definite morphological structure has been required as indispensable for an admission of differences in the stem-cells. One has but to remember the elaborate, but vain efforts of Schridde to put in evidence a difference between the leucoblasts and the lymphoblasts.

In order to avoid somewhat irrelevant details, I shall take as support for my position the meroblastic eggs of reptiles and birds. All the blood cells of these animals are real cells, containing both cytoplasm and nucleus. This fact is not unimportant for a true conception of the principle of haematopoesis. The statement that the erythrocytes arise in the early stages of embryonic development from the blood-islands cells in the area opaca has remained unshaken. Recent studies of the origin of the blood islands has established definitely their mesodermic origin. The mesoderm contributes to the development of both,—the blood-island cells and the endothelium. The mesenchymal cells of the mesoderm (*MSCH.*) appear to be a source from which develop various specific differentiation products depending on the gradual assertions of the environment.

We may, with our present knowledge, define at least some of the conditions necessary for an arrangement and differentiation of the mesenchyme into haematopoietic organs (namely, those comprising the differentiation of red and granular cells). The area opaca, as is known, lies above the yolk, and the connection between the development of a haematopoietic organ and an abundance of nutritive material is still more obvious if the results of studies on the development of other haematopoietic organs are compared therewith.

Still another condition seems to be of equal value to the above. This consists of the localization of the developing haematopoietic organ in a place, where neither extensive growth, replacement of different organs nor muscle contraction takes place. Only in such regions of the embryo body will the mesenchyme under normal conditions develop into a haematopoietic organ, always characterized by a highly developed network of capillary veins.

The formation of the blood-islands is accompanied by the development of an endothelial membrane. A vascular wall surrounds the blood-islands, regardless of the fact that a communication with the heart may or may not be effected (Loeb, Stockard). I must emphasize here that the endothelial capillaries develop not only around the blood-islands, but also in regions where these are lacking, and that some of the blood-islands remain outside of the haematopoietic capillaries.

Just before the differentiation of the endothelial membrane takes place, all the cells of the blood-islands have a similar structure (*BLISC. (HBL.L.LMC.)*), regardless of whether they appear as aggregates of numerous cells, merely as small groups or even as isolated cells. These cells possess an intensely basophilic, slightly vacuolized cytoplasm, a spherical nucleus with a well pronounced nucleolus, and they are potentially spherical, but very amoeboid in character. This structure, characterizing, as it does, the stem-cells of the blood elements from the earliest stages of their appearance, remains unchanged in the development of new haematopoietic organs (compare *EV.HBL.*, *IV.HBL.* and *HBL.* in I, II and III).

The differentiation of the endothelial membrane (I, *ENDC.*) separates the major portion of the blood-islands from some of these cells, as well as from other amoeboid cells, which still continue to split off from the mesenchyme (I, *EV.HBL.*). This separation of similar elements establishes very different environments for different groups of cells. The physical and chemical conditions within the vessels, which are lined with a continuous endothelial membrane, must differ largely from the conditions offered by the tissue outside the vessels. This difference in environmental conditions necessarily affects the further development of previously like stem-cells. However, the reproduction of the stem-cells is evidently not influenced by the different environmental conditions, for the mother cell multiplies within as well as outside the vessels (I, *EV.HBL.*, *IV.HBL.*). The differentiation, on the contrary, is subject to strong influences of the environment and manifests itself in very different ways. Within the vessels the stem-cells produce in their cytoplasm haemoglobin (I, *ERBL.*), outside of the vessels the same young undifferentiated cells give rise to acidophilic granules (I, *GRBL.*). Changes in the nuclear structure keep pace with the differentiation in the cytoplasm.

In the early development of the blood cells in reptiles and birds (Danchakoff), and partly in fishes (Maximoff), many similar features may be noticed during the simultaneous differentiation of the granuloblasts and erythroblasts from a morphologically and genetically identical mother-cell. This stem-cell maintains its own existence (I, *EV.HBL.*, *IV.HBL.*) by uninterrupted multiplication, on the one hand, and on the other it differentiates into red cells (*ERC.*) within the vessels and into granular leucocytes (*LCC.*) outside the vessels.

The first development, differentiation and multiplication of the blood cells occurs in mammals (Maximoff), birds, reptiles and in some fishes on the surface of the yolk. As is known the fundamental difference existing between the eggs of reptiles and birds and those of mammals, consists in the fact that the latter have lost the yolk so characteristic of the eggs of reptiles and birds. This fundamental difference necessarily requires a difference in

the development of the haematopoetic organs in these groups of animals.

The rapid appearance of anlagen and the development of different organs in the embryo body naturally attracted the attention of the embryologists to the body of the embryo itself. This fact accounts for a comparatively early recognition of the haematopoetic function of the liver. The first development of the blood cells in the area vasculosa, the embryonic localization of the haematopoiesis in the liver and its final establishment in the bone marrow,—such are the data fixed for the erythro- and granulopoiesis in the mammals.

The most minute and laborious study of the whole embryo body in birds has failed to show the localization of the erythropoiesis before a comparatively late stage. By the 13–14 days of incubation it has started in the bone marrow. A cursory glance at the liver of the meroblastic eggs was sufficient to establish an absence of any noticeable blood development in this organ. Neither the thymus, the spleen, nor the tissue of the Wolffian body (mesonephros) shows any indication of erythropoiesis. All these organs contain vessels and capillary nets but they are all merely thoroughfares.

Although in regions far removed from the embryo body in the annexes of the yolk-sac there was demonstrated by injection (Popoff) an amazingly extensive and exuberant net-work of large venous capillaries, its development and existence seemed to be fully explained by the resorption of the enormous quantity of yolk, and no one thought of thoroughly analyzing the cell elements present within the capillaries. Whereas a closer study (Danchakoff) of this capillary net and of its contents would show that the large meshes of this net are distended by young undifferentiated blood cells, and it would be easy to observe that outside of the capillaries the basophilic amoeboid cells are also rather numerous. The structure of all these cells, both within and without the capillaries would permit of ascribing to this capillary net a real haematopoetic (erythropoetic and granulopoetic) function, homologous to that of the mammalian liver.

Both within and without the vessels the young mother-cells bear the same structure as the blood-island cells after their isolation (I, *EV.HBL.*, IV. *HBL.*). This morphological cell unit was most unhappily termed the large lymphocyte. The efforts of the last 7 to 8 years have not yet been able to render conclusively the dictum, that the morphological cell unit, formerly known as the large lymphocyte, appears first by the dissociation of the blood-islands and is a young, undifferentiated cell. This cell differentiates in accordance with given environmental conditions. Briefly, it corresponds morphologically to the large lymphocyte and is physiologically the haemoblast.

It appears only natural that the young stem-cells, observed in the haematopoietic net of the yolk sac annexes, corresponds exactly to the stem-cells which were first found in the blood-islands; for they appear to be the direct result of an uninterrupted reproduction of the latter.

The erythropoiesis and the granulopoiesis now remain distinctly separated, as in the first stages of their development. The distribution of the different cell elements within the haematopoietic capillary net in the annexes is always very characteristic. Every cell is free and does not manifest any continuity with its neighbor. The cells lie very closely together in the zones adjacent to the endothelial membrane, and their reciprocal pressure flattens them and renders them polygonal. Even a slight fluid current could hardly be supposed to be present in these parts of the vessels. In the centre of the vascular lumen the cells are arranged more loosely and are free from pressure. The position of the cells shows clearly, that they are removed from here and directed by the current into the body of the embryo.

It is easy to notice, that the most highly differentiated cells, rich in haemoglobin, lie in the centre of the vascular lumen, just where the current is strongest. Besides these already differentiated cells the capillaries include numerous cells in various stages of differentiation, beginning with the youngest haemoglobin-free stem-cells, and including the whole range of cells which gradually accumulate haemoglobin in their cytoplasm and equally

gradually change their structure into that characteristic of an erythrocyte.

The youngest stem-cells, which I will call lymphoid haemocyto-blasts, are found in the zones immediately adjacent to the endothelial membrane, and these young blood cells within the vessels are so much like the white blood cells lying outside the vessels, that Bizzozero regarded them in the bone marrow as a layer of white blood corpuscles. In the embryonic haematopoietic organs these cells are very numerous and sometimes form a continuous layer of basophilic cells, becoming gradually substituted towards the centre by cells, which accumulate haemoglobin in their cytoplasm.

The structure of the haematopoietic net and the distribution of the cells in their different stages of development reminds one strongly of the conditions under which spermatogenesis takes place. Here is seen the same uninterrupted differentiation of the younger cells into highly differentiated cells, which thereby lose much of their usual cell appearance. We also find the same location of the young undifferentiated elements, which secures their existence by uninterrupted multiplication and continuous splitting off of cell generations which undergo further differentiation. Finally, we see the presence of a current in the centre of the vessels, which removes the differentiated products.

As mentioned above, the area vasculosa of birds and reptiles contains outside the vessels numerous cells, which give rise to the granular leucopoiesis. Both in the earliest stages and now in the yolk sac annexes the leucocytes develop extravascularly. They nevertheless show very close connection with the vessels, occasionally surrounding them by a continuous layer. Their differentiation here follows the same principles as elsewhere and from a common morphologically and genetically identical stem-cell (I, *EV.HBL.*) leads to the development of numerous special (acidophilic) leucocytes (I, *LCC.*). The differentiation of blood cells in the area vasculosa and in the annexes of the yolk sac is completed by the development of the cell elements described.

Another question now arises, viz., the question of how a new haematopoietic organ originates, whether it is the mammalian

liver or the definitive haematopoetic organ—the bone marrow, or finally, one of the organs assigned to the lymphopoesis.

I have mentioned above how easily this problem was solved not long ago. The first red cell (the erythroblast) differentiated from the blood-island cell; it supplied the organism, by multiplication and differentiation, with erythrocytes. At a given time the current transported the erythroblasts into definite regions of the embryo body. They stopped there, multiplied and differentiated further. Shortly the development of a new haematopoetic organ appeared as a strictly localized metastasis. It was difficult even to expect any other conclusion from studies made upon haematopoetic organs, the development of which was more or less advanced. Indeed, such organs offer a complete parallel between the differentiating blood cells in the new organ and those, which were observed in the earlier organ. Only studies of the very earliest stages in the development of a new haematopoetic organ will show by definite evidence, that the development of a new organ is due, not to metastasis from a former existing organ, but to a local differentiation and multiplication of the mesenchymal cells, still preserving their faculty of polyvalent differentiation (see II and III).

The young undifferentiated mesenchymal cell (*MSCH.*) maintains itself for many days, if not for the whole duration of the life of the organism, and evidently retains its potency for multiplication and differentiation. Similar mesenchymal cells give rise to accumulations of amoeboid cells around the embryonic double aorta, described by Professor Huntington. The blood-island-like agglomerations of similar cells in the development of the bone marrow arise also by a splitting off from the mesenchyme. An endothelial vascular membrane, partly formed *in loco*, partly growing from outside the bone marrow cavity, surrounds some of these cell groups and marks them at the same time as erythroblasts. Similar cells, remaining between the capillaries, become the source of the granulopoesis (Maximoff, Danchakoff).

That being the case, we have to conclude, that the whole development of the new haematopoetic organ f. ex. of the bone marrow (see plate III) takes place locally and leads to the double

differentiation of a common mother-cell into red and granular cells, the young stem-cell itself being most tenaciously retained in the precincts of the haematopoetic organ.

Nevertheless, it is possible to notice certain features of the haematopoiesis in the bone marrow, characteristic for this organ, which appear already in the embryo and are very conspicuous in the adult animal. This is a reduction in number of the young undifferentiated stem-cells, both in the erythropoiesis and the leucopoiesis regions. The development of the highly differentiated erythrocytes and leucocytes is effected by the further development of cells which already contain respectively haemoglobin (erythroblasts) or acidophilic granules (granulocytoblasts). The haematopoiesis now develops in a more homoplastic way, differing from that seen in the early embryonic stages in which the haematopoiesis develops principally in a heteroplastic way. This difference is, however, not essential; one has but to bleed the animal, whereupon the stem-cell promptly produces by multiplication a whole generation of similar stem-cells (Danchakoff), which are very numerous in the haematopoetic tissue of the embryonic organism.

Some essential differences may still be observed in the structure of the bone marrow, in comparison with the former haematopoetic organ, namely new directions in the differentiation of the blood cells, leading to the development of small lymphocytes and of Mast cells. Since the latter find their origin on a larger scale in the connective tissue and in special organs, lymph glands, thymus and spleen, the mode of their differentiation is more conveniently studied in the latter organs.

Finally, I have to emphasize the striking analogy seen in the general conditions which accompany the development of every new erythro-granulopoetic organ: an abundance of food supply and the localization distant from any rapidly growing organs; these conditions are met with both in the yolk sac and in the cavities of the bones and seem to be so essential that in reptiles which have no extremities, haematopoiesis finds its localization in the cavities of the vertebra, thus developing a haematopoetic organ in the form of a whole series of isolated centres

(Danchakoff). Even the localization of haematopoiesis in the liver of mammals, which have lost the yolk in their eggs, correspond in the highest degree to the conditions cited and which, at this time, the embryo offers.

Some of the environmental conditions for the differentiation of the mesenchyme into an erythro-leucopoetic organ, as well as those for the development of a haemocytoblast into an erythrocyte or into a leucocyte could be, at least partly, explained. However, the differentiation of the blood stem-cells into erythrocytes and leucocytes does not present all the possible inherent potentialities of the haemocytoblast. New differentiation products result under new conditions in the mesenchyme of the embryo body (see plate II).

The mesenchyme cells are disseminated through the whole organism and occupy all the interspaces between various organs. It is difficult at present to define the conditions under which the mesenchymal cell undergoes differentiation into a fibroblast. This ubiquitous differentiation has for long overshadowed, by its omnipresence and its conspicuousness, all the other lines of development which find place in the so-called connective tissue. Meanwhile certain other directions in the differentiation of the mesenchymal cells are almost as general as the differentiation into the fibroblasts; they convert the connective tissue into a seat of most interesting diffuse lymphopoetic, and, in early embryonic stages and in lower animals, into granulopoetic differentiation. The development of the lymphatic glands, of the thymus and of the spleen thus becomes merely localized hypertrophic centres of a similar diffuse differentiation, spread out through the loose connective tissue.

In speaking of the lympho-granulopoesis, I therefore associate the differentiation phenomena in all the regions above mentioned in one common account. These lines of differentiation are very simple and similar. The first isolation of the mesenchymal cells from the general ties takes place as a rule in the form of large lymphoid haemocytoblasts (II, *HBL.*, *L.LMC.*), which sometimes arrange themselves as blood-island-like groups of cells. Their rapid, uninterrupted multiplication produces numerous smaller

cells (II, *SM.HBL.*). These cells become a source of the true small lymphocytes (II, *SM.LMC.*) and of numerous wandering cells (II, *W.C.*), so characteristic of the loose connective tissue. The latter may also arise directly from the mesenchymal cells, especially in later embryonic stages. With the appearance of the small lymphocytes and wandering cells the differentiation possibilities of the lymphoid-haemocytoblast in the connective tissue are still not exhausted. Throughout the regions of the embryo body in birds and especially in reptiles, but less evidently in mammals, the splitting off of granuloblasts is very conspicuous (II, *GRBL.*). Most of them contain acidophilic granules. The differentiation of the mast-cells with basophilic, metachromatic granules at the expense of the mesenchyme cells takes place in the later embryonic stages.

For a long time the ability of the small lymphocytes to multiply by mitosis was denied; at the present their reproduction by mitosis is fully admitted, as well as their faculty of further differentiation into mast-leucocytes and into specific granulocytes with spherical acidophylic granules, very numerous in the connective tissue of birds (II, *GR.LMC.*). Likewise they may differentiate into plasma cells in the adult organism (II, *PLC.*)

The question of regeneration of the blood cells in an adult animal is a very important problem and the embryological data above described may contribute directly to its elucidation. The question of blood regeneration has interested most keenly the pathologist-clinicians. Facing the various highly differentiated products (*LCC.*, *ERC.*, *PLC.*, mast-cells, etc.) which, as above mentioned, develop in the adult animal chiefly at the expense of cells in intermediate stages of differentiation, the pathologist-clinicians could not but admit a polyphyletic origin for various blood cells. The red cells possessed their own stem-cells, as also the different leucocytes and lymphocytes.

This statement is not altogether wrong; inasmuch as referring to haematopoietic organs in an adult organism, it solves practically the problem of the usual haematopoiesis. But this statement does not cover all the eventualities. Regeneration after bleedings, as well as the excessive increase in number of white

blood corpuscles in leukemias, takes place not at the expense of specific granulo-lympho- or erythroblasts. The monophyletic school has the credit of demonstrating in all the haematopoetic organs, in the bone marrow, in the spleen, in the lymph glands and in the loose connective tissue, the presence of a similar morphologically and genetically young and undifferentiated stem-cell, at the expense of which the regeneration really takes place, especially after great loss (Pappenheim, Weidenreich, Maximoff, Danchakoff). This young stem-cell bears the same structure as the morphological cell unit, which was recognized by the monophyletic school as being the mother-cell of all the different blood cells in the development of every haematopoetic organ.

I am afraid to be too positive, but there are some indications that after extensive destruction of the haematopoetic tissue regeneration occurs, at least in some cases, not only at the expense of haemocytoblasts, but also at the expense of undifferentiated mesenchymal cells, retaining from the very beginning of their development all their polyvalent potentialities. This would mean that the adult organism retains, from the remote period of its embryonic life, a stock of undifferentiated mesenchymal cells which begin to multiply and to differentiate in certain conditions of a disturbed equilibrium. This latter question is in a stage of development. No matter how this question may be solved, the similarity of the morphological structure of the stem-cell in all the haematopoetic organs and its common origin from the mesenchymal cells of the mesoderm in embryonic life demonstrates the close relations existing between different blood elements.

It would not follow that the various differentiation products are not different, or that they had no different functions. The erythrocytes, the small lymphocytes, the different leucocytes, the wandering cells of the connective tissue, the mast-cells and the plasma cells,—all these cells are different cell units, morphologically as well as physiologically. But in the early embryonic stages they all had a common mother-cell, and this mother-cell is preserved in the adult organism and becomes the source of differentiation and regeneration and most probably also the source of pathological proliferation.

The conception of the monophyletic school has recently been subjected to searching criticism. As I, in great measure, uphold these conceptions, I feel obliged to plead their cause.

Embryos without blood circulation were obtained by means of the influence of alcohol on the fertilized eggs of *Fundulus* (Stockard).² Blood-islands developed on the yolk surface, and endothelial membranes surrounded them; the blood-island cells developed into haemoglobinic cells; but they were never carried into the embryo body, because the connection between the heart and these vessels failed to become established. During the summer of 1914 I personally observed precisely the same conditions in hybrids of *Fundulus* and *Menidia*, which were obtained for this purpose at the kind suggestion of Dr. Loeb.

The fact, that the blood-island cells develop into erythrocytes in embryos without circulation, is accounted for by a specific character of the mesenchymal cells, which differentiated only in haemoglobinic cells. But it is stated that no leucocytes are developed on the yolk sac in normal *Fundulus* embryos.

The specificity of the stem-cells of the white blood corpuscles is based upon observations of small groups of cells "which resemble lymphocytes or leucocytes;" it is, moreover, stated that "these embryonic cells all show a more or less degenerate appearance, but if they can be classed as any type of white blood cell, their origin is definitely removed and entirely distinct from that of the red blood cells."

These are some of the data, upon which an attempt was made to build a new embryopolyphyletic school.

The experimental analysis of the problem of haematopoiesis is undoubtedly of very great importance. I am fully conscious, how important is the study of embryos without circulation and with disconnected vessels for a true conception of the development of vessels. But I fail to understand how the study of these embryos, on which the polyphyletic origin is now based, can help us in understanding the development of different blood cells and of haematopoietic organs.

² The origin of blood and vascular endothelium in embryos without a circulation of the blood and in the normal embryo. *Amer. Jour. Anat.*, vol. 18, no. 2, Sept., 1915.

Whether the circulation is established or not, the differentiation of the blood cells follows the same principles. How can the presence of the circulation embarrass the study of the development and differentiation of the blood cells in the haematopoietic organs, if only more or less differentiated cells are withdrawn by the circulation? It seems to me that the absence of circulation in itself must greatly obscure the study and hinder the true understanding of the processes. Indeed, the differentiated products are not withdrawn in embryos without circulation, they remain *in loco* and evidently influence very strongly the further reproduction and differentiation of young cells, for finally they all disintegrate.³

The environmental conditions are also taken into account by the supporters of the theory of the polyphyletic origin of the blood cells; but only for the multiplication of the erythroblasts, not for their differentiation. In spaces, lined by endothelium, the erythroblasts do not multiply in the *Fundulus* embryos. If this statement, drawn from the study of *Fundulus*, was not generalized, we had to believe, until further proof, that the erythroblastic tissue of *Fundulus* is a very peculiar tissue, refusing to multiply inside the vessels. In birds and reptiles and in some cartilaginous fishes (Selachians) the erythropoiesis takes place exclusively inside the vessels. Mitosis in erythroblasts inside the vessels are as numerous, as mitoses in granuloblasts outside the vessels. On the contrary, the differentiation of the cells is strongly influenced by the fact, whether the cells lie inside or outside the vessels.

But let us consider some of the arguments against the monophyletic school. A special value is evidently assigned to the following argument, for I find it quoted in different places in the paper. "If all the types of blood cells did arise from a common mother mesenchymal cell, they should then be found in intimate association throughout all blood-forming regions."

³ Analogous results of disintegration of spermatogenetic cells are offered by the interesting studies of B. D. Myers. The disintegration of the undifferentiated spermatogenetic cells is caused in his experiments also by a lack of withdrawing of the differentiated cells. See: Histological changes in testes following vasectomy. Proceedings of the Am. Assoc. of Anat., 1916.

The conclusion cited can only appear as a sequence to an assertion, stating that various differentiation products, developing at the expense of a common stem-cell, must always be found in intimate association. But this assertion is not correct.

If various products of differentiation have their origin in a common stem-cell, they must be found under the influence of different environmental conditions which separate them. If various products of differentiation are found in absolutely identical environments the difference between them may be explained only by differences in their stem-cell.

Imagine in the remote past a heap of similar tree seeds. These seeds develop in our moderate climate into tall and many branched trees. Suppose the wind bears a part of the seeds away and brings them to a land possessing different environmental conditions, we will say to arctic lands. There the seeds may develop but they may produce trees no higher, than our moss (willow-salix in Spitzbergen). Have we the right to say that the difference between the various products of seed development are caused by differences in the seeds? They may be different or similar; morphologically they were similar.

How would it be possible to know if there were differences in the seeds? The only possibility of solving this problem would consist in sowing the seeds of arctic trees in our climate and vice versa. If the seeds of our tall trees again produce dwarf trees in the arctic lands, the seeds of the different products of development will have been shown to have been identical.

Similar experiments may be carried out with the haematopoietic tissue. The experiments are made the more possible, because of the short time periods, needed for the cell differentiations, and fully controlled by us.

It is difficult to assert at present, whether or not the monophyletic school will contribute the last word in the establishment of the mutual relationship and of the origin of the blood cells. The possibility is not excluded that perhaps some time we will find direct or indirect means of distinguishing between different mesenchyme cells, but there must be other arguments than those cited above to convince one of the truth of the position taken by

the polyphyletic school. However, the fact that the stem-cells, as stem-cells, keep so tenaciously under the most varied conditions their morphological structure and that only their products differ, seems to speak strongly for the existence of a single stem-cell, identical in the different haematopoetic organs.

Through the kindness of the Rockefeller Institute for Medical Research I have been enabled to include a colored plate, illustrating the course of the gradual differentiation of various cells in the haematopoetic organs.

PLATE 1

EXPLANATION OF FIGURES

The plate shows the development and the gradual differentiation of the blood cells.

I Erythro- and granulopoesis in the yolk-sac of birds.

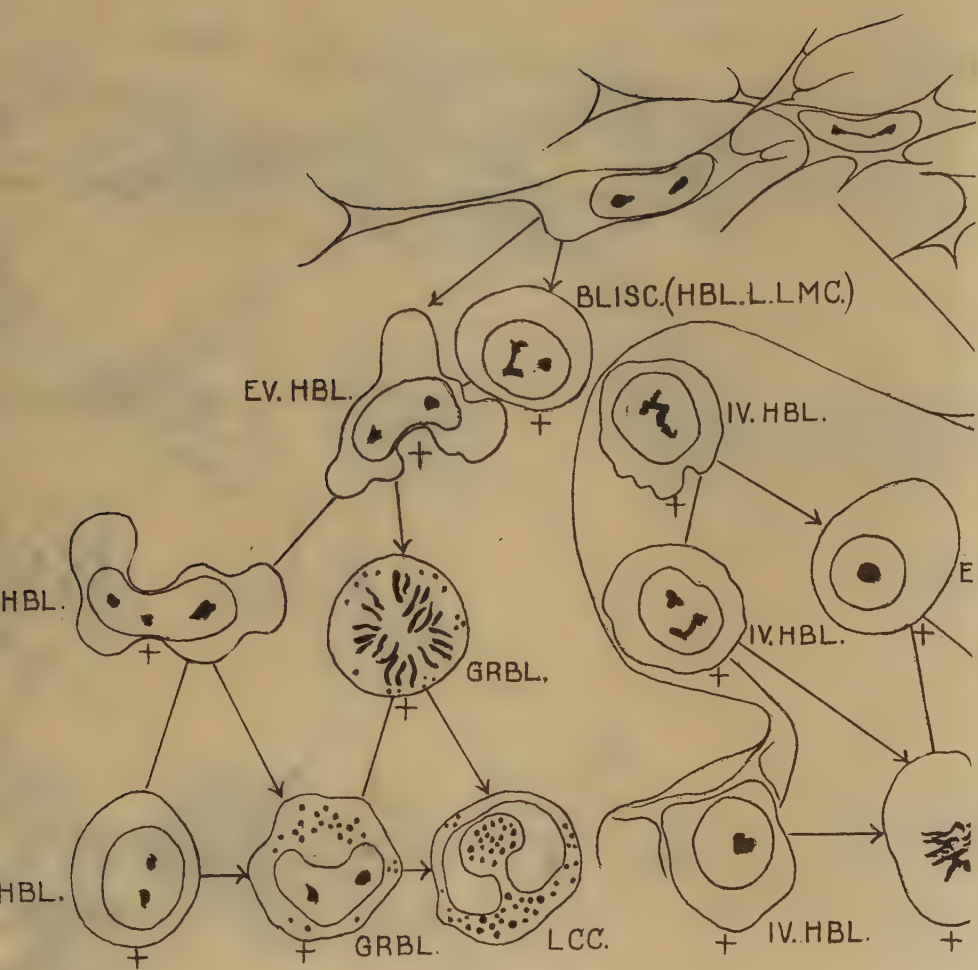
II Lympho- and granulopoesis in the special lymphatic glands and in the connective tissue.

III Starting point of the development of the erythro-granulopoesis in the bone-marrow, which proceeds, as is shown in *I*.

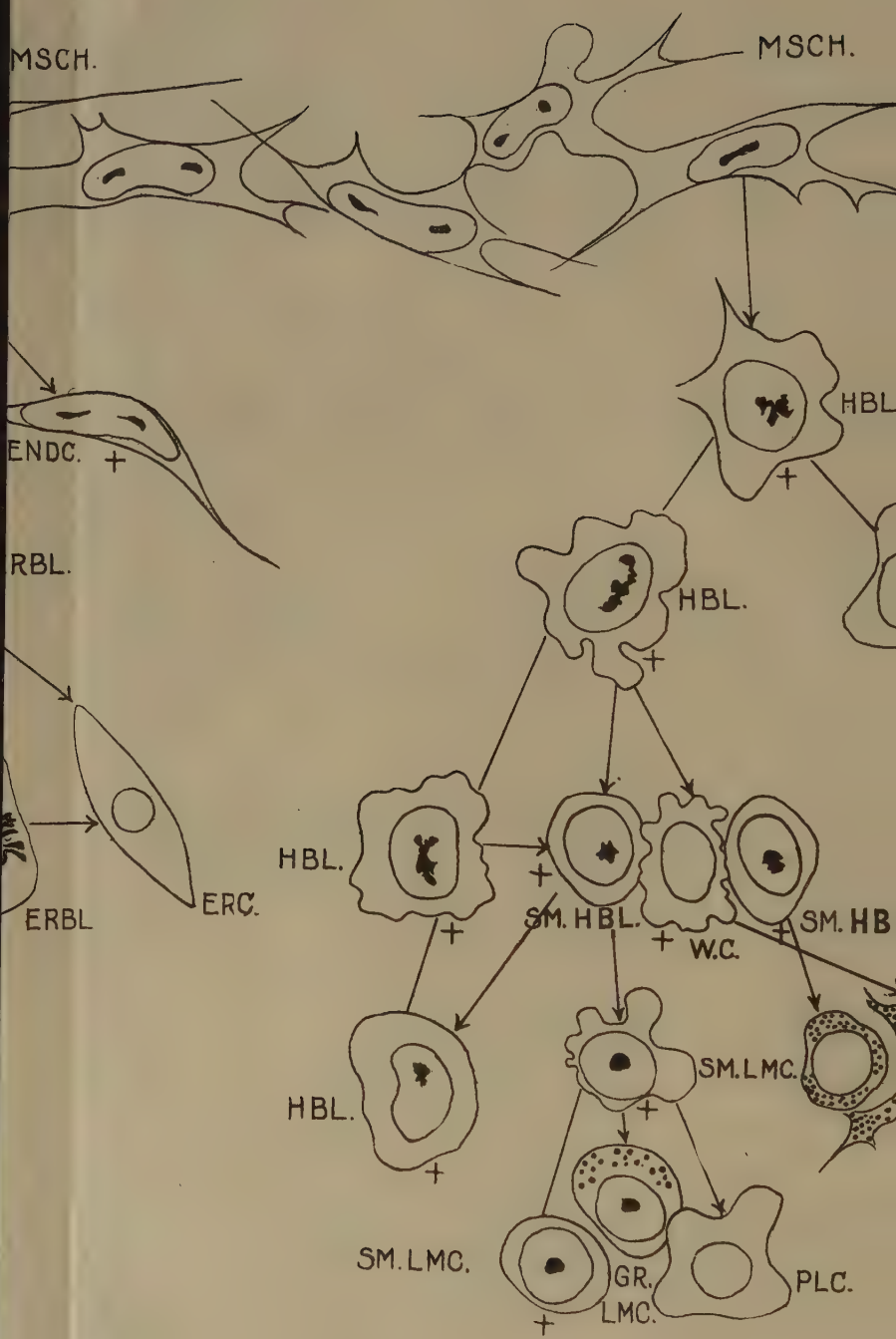
Arrow—denotes differentiation. Simple line—denotes multiplication. Cross—indicates cells, capable of mitosis.

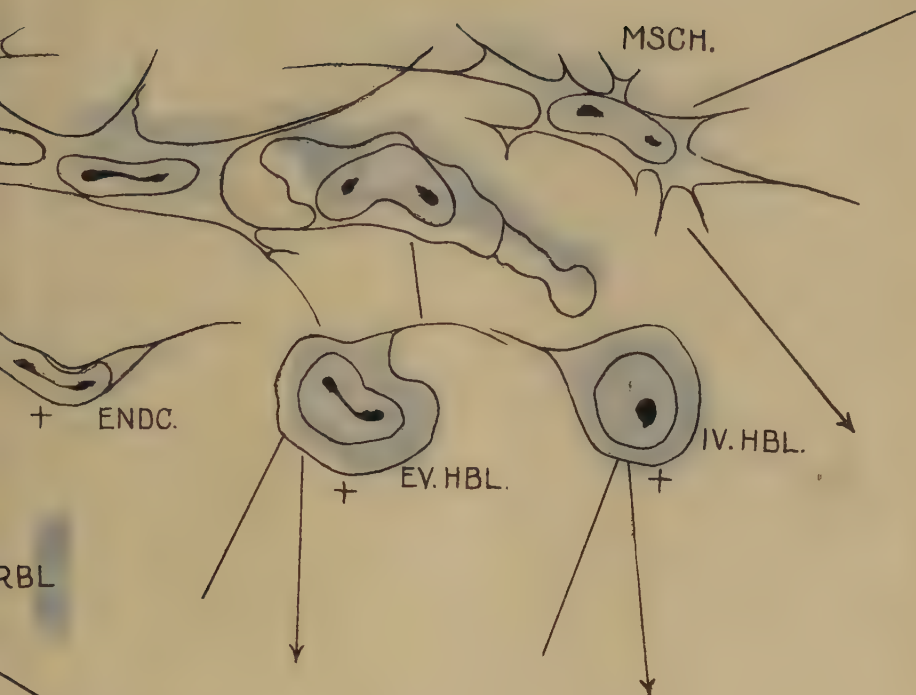
ABBREVIATIONS

<i>BLISC.</i> (<i>HBL.L.LMC.</i>), blood island cell (haemoblast) large lymphocyte	<i>HBL.</i> , haemoblast
<i>ENDC.</i> , endothelial cell	<i>IV.HBL.</i> , intravascular haemoblast
<i>ERBL.</i> , erythroblast	<i>LCC.</i> , leucocyte
<i>ERC.</i> , erythrocyte	<i>MSCH.</i> , mesenchyme
<i>EV.HBL.</i> , extravascular haemoblast	<i>PLC.</i> , plasma cells
<i>GRBL.</i> , granuloblast	<i>SM.HBL.</i> , small haemoblast
<i>GR.LMC.</i> , granular lymphocyte	<i>SM.LMC.</i> , small lymphocyte



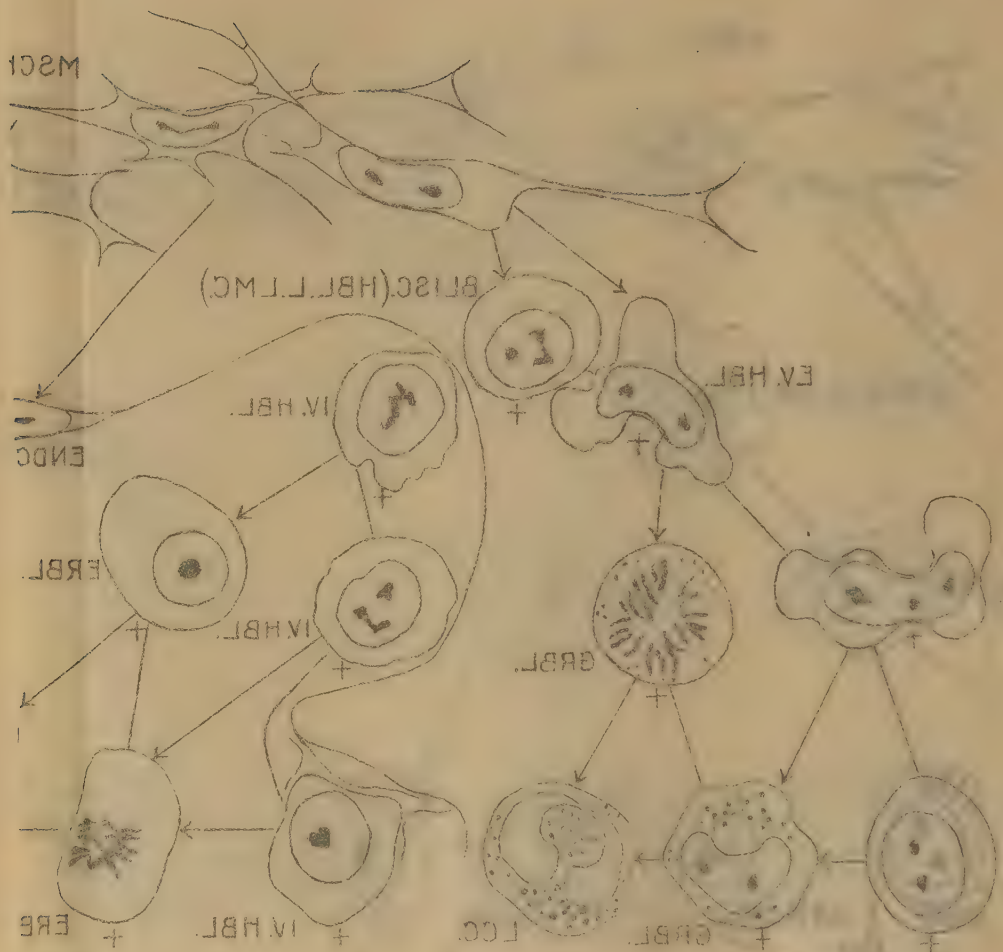
I





III





I

ORIGIN OF THE BLOOD CELLS
VERA DANCHAKOFF





CONCERNING THE CONCEPTION OF POTENTIALITIES IN THE EMBRYONIC CELLS

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The study of a complex and peculiar tissue, such as blood, requires great care in the methods used and in the conclusions drawn. It seems especially desirable to establish the limits to our knowledge imposed by the actual methods.

The interesting article of Dr. Stockard, appearing in *Science* October 15, seems to reject the limitations of morphological study.

If two tissues behave differently in their development, the cause thereof, according to Dr. Stockard, lies in the difference of their anlagen, and even if no morphological differences can be traced in these anlagen, they exist potentially. These anlagen must be different, since the products of their development are different and the conditions of their development do not differ enough to account for the various products of the development.

Dr. Stockard discusses in his article the development of erythrocytes, endothelial cells and two kinds of chromatophores found in the yolk sac of the bony fish *Fundulus*; though he admits that they develop from "apparently similar mesenchymal cells," yet he concludes, that "these four types of cells" must be considered "as being polyphyletic in their origin."

The conclusion, concerning the potential difference of the mesenchymal cells, is based upon statements: 1) that similar mesenchymal cells develop in different ways; 2) that the four types of cells, resulting from the differentiation of the mesenchyme cells in the yolk sac cannot 'intergrade' nor 'transmutate.'

As to the statement of the non-transmutation of the differentiated cells, it is fully supported by facts, but is useless as an argument, for no one has endeavored to affirm that the chromatophores may differentiate into any other type of cells: they may only multiply. They have lost the faculty of splitting off undifferentiated cell generations, by reason of their highly specialized nature; they are merely able to repeat their life cycle between each two mitoses.

The life history of the endothelial cells is somewhat more complex. These cells, at least in the very early embryonic stages, are only slightly differentiated in birds and, according to Maximoff, in mammals; therefore they in part keep their potentiality for polyvalent development. One may contradict this fact. But even if the mesenchymal

cell as an endothelial cell really loses its faculty of independent existence and further development, it does not necessarily follow, that the mother cell of the endothelium must bear in its structure a constitution, which will with absolute necessity make it only an endothelial cell and not an embryonic connective cell nor even an undifferentiated blood cell. The conditions, in which the one or the other mesenchyme cell may be found, cannot be immaterial in directing their development;—and the data found by the study of the yolk-sac of the birds and the reptiles speak strongly for a dissimilarity of conditions.

The non-transmutation is not a proof of a polyphyletic origin: *a* and *b* may never transmutate, but may present two different offshoots of a common stem. Dr. Stockard is inclined to see this common stem one generation back, without giving any material basis to support this view.

Moreover if the mere differentiation of cells leads one to admit a difference in the stem cells, the admitted differences in the stem cells must necessarily compel a conception of differences in the ancestors of the mother cells, and so on.

All these assumptions have logical evidences only in case the different products are obtained *ceteris paribus*. The conditions of the environment in the yolk sac of fishes are supposed by Dr. Stockard to be identical for all the cells. A certain degree of uniformity (or an absence of special conditions) in the yolk sac of fishes may account for the absence there of leucocytic development. (Dr. Stockard did not find leucocytes in the yolk sac of *Fundulus*, while in the yolk sac of reptiles and birds the leucocytes and their stem cells are very numerous.)

Very definite data obtained from a study of the development of the blood in the yolk sac of the reptiles and birds, urge us to postulate a potent influence of different conditions upon the differentiation of the primitive blood cells in various directions. The peripheral cells of the larger blood islands invariably differentiate into endothelial cells; the basophilic amoeboid cells of the blood islands invariably differentiate within the vessels into red blood corpuscles, preserving at the same time their stock by continuous multiplication. Some of the smaller blood islands between the vessels, as well as numerous other cells, isolated later from the mesenchyme, differentiate only into granular leucocytes.

The reference of various processes of differentiation to potential morphologically indefinable differences in the stem cells seems to me a source of danger, because similar assumptions are apt to impede further investigations. They put aside the important question,—“What are the conditions and the causes for the development of certain groups of morphologically identical mesenchyme cells into morphologically different products of differentiation?” Until our resources in methods of investigation are exhausted, a reference to invisible and indeterminate potential differences, as bases for cell differentiation, would seem to be illogical.

EVIDENCE OF HEMOGENIC CAPACITY OF ENDOTHELIUM

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The yolk-sac of the 10 mm. pig embryo is an active source of hemoblast origin from endothelium. The evidence of this process consists in a complete series of transition stages from primitive endothelium to definitive erythrocytes (*Anat. Rec.*, vol 9, p. 92). Similar evidence accrues from a study of the capillaries and smaller blood vessels immediately next the embryonic brain and spinal cord, and of the hepatic and mesonephric sinusoids. Certain objections can be made to the interpretation of appearances here given, most forceful of which is the one that hemoblasts of various stages of development at various phases of transit through an incomplete endothelial wall of irregular contour give the deceptive appearance of endothelial origin. (Stockard, *Am. Jour. Anat.*, vol. 18, p. 592). To meet this objection is the main purpose of this paper, other objections as concerns the yolk-sac vessels being elsewhere considered.

In searching through the 10 mm. pig embryo for intrasomatic evidence of giant cells in connection with my study of these cells in the yolk-sac, my attention was arrested by the presence of peculiar cell clusters in the aorta. Brief attention has been called to these also by Emmel (*Anat. Rec.*, vol. 9, p. 77). He describes them for pig embryos between 6 and 15 mm. length and in rabbit and mouse; and I have seen them also in mongoose and turtle embryos. Their occurrence would seem to be quite general in young vertebrate embryos. It is from a study of these clusters that I believe the most cogent evidence accrues of intrasomatic hemogenic capacity of young endothelium; there is here no question of a deceptive appearance due to lodgment in an endothelial bay or lacuna, and a peculiar plane of section.

Here we may note the difficulties of presenting decisive proof that endothelium transforms into blood cells. The morphologic evidence must consist in a complete series of developmental stages between an endothelial cell and a separating primitive blood cell. But the critic can always object up to a certain point that the particular cell in question is not a blood cell; at a later point he can object that the cell, apparently separating from the endothelium, is simply a hemoblast in intimate contact with the endothelium due to pressure and the adhesive properties of protoplasm. This is why Stockard (*Am. Jour. Anat.*, vol. 18, p. 229) can brush aside all the morphologic data as unsatisfactory, in

the light of his experimental findings in the narcotized *Fundulus* embryo where the endothelium is proved to have no hemogenic function. In the case of individual cells the supporter of endothelial hemogenesis is apparently helpless, even with a complete series of drawings, at the hands of the dissenter. The cell clusters of the aorta appear to be ideally constituted to meet all possible objections as to the hemogenic capacity of primitive endothelium.

These cell clusters appear in the aorta only throughout the region of the mesonephroi. They are limited to the ventral portion of the wall. They may consist of few or of many cells. At certain levels two clusters appear, one on either side of the mid-line. Proximally they are intimately associated with the endothelium; in certain clusters true endothelium appears to be entirely lacking beneath the mass of primitive blood cells. Passing distally, transition stages appear between endothelium and hemoblasts. Occasionally cells show mitotic and amitotic division phenomena. The following gives a résumé of the occurrence of aortic blood cell clusters in a 10 mm. pig embryo:

Scattered cells first appear in the mid-ventral portion of the aorta in slide no. 22; this is 5 slides caudal to the cephalic tip of the mesonephros, at the point of division of the dorsal aorta into the paired aortae.

Slide no. 26.... 4 groups of 3 or 4 sections (10 microns) each.

27.... a) cluster passing through 10 sections.

b) a double group of 4 sections.

28.... cluster of 4 sections.

(also scattered cells in a ventral branch—coeliac axis)

29.... cluster of 8 sections.

30.... cluster of 4 sections.

31 and 32.... cluster of 13 sections. (130 microns, including several hundred cells: also scattered cells in ventral branch—superior mesenteric artery).

33.... cluster of 11 sections.

34.... 3 groups of 4, 5 and 4 sections respectively.

35.... 2 groups of 3 sections each.

37.... a group of 3 sections.

Here the following points must be emphasized; 1) similar clusters are found nowhere else, either in the yolk-sac or the embryonic vessels or sinusoids, not even in the aorta or its branches, outside of the mesonephric area; 2) just cephalad of the first cluster the endothelial cells of the ventral portion of the aorta for some distance are short stout spheroidal elements, with early hemoblast cytoplasmic and nuclear characteristics, similar to the hemoblast transition stages described for the yolk-sac vessels; 3) from the ventral surface of the aorta, where the clusters are located, numerous median and ventro-lateral (mesonephric) branches arise, an occasional endothelial cell of which has the same spheroidal shape and early hemoblast characters; 4) the clusters are not caught in the mouths of these vessels but generally lie between the openings of such vessels; occasionally also a small cluster lies freely suspended attached to the side (outer) of the ventro-lateral branch.

The interpretation I wish to maintain regarding these cell clusters, to which also Emmel seems to incline, is that they arise from the endothelium by process of proliferation and differentiation. Two main objections must be met: 1) that these cell clusters, presumably derived from endothelium, do not consist of primitive blood cells; 2) that as clusters of hemoblasts they are only spatially not genetically related to the endothelium; that they are groups of developing blood cells swept toward the ventral wall of the aorta by the stronger currents towards the numerous ventral branches and caused by pressure and their adhesive properties to adhere to the ventral wall of the aorta.

To the first objection the countervailing fact may be stated that according to all the criteria both cytoplasmic and nuclear, gathered from a study of isolated hemoblasts and erythroblasts in the yolk-sac vessels, liver and heart of the embryo, the majority of these cells are either hemoblasts or erythroblasts.

Against the second objection the following facts may be cited: 1) The cells frequently show a series of transition stages from endothelium to hemoblasts in passing from the attached pole to the free periphery; 2) the fairly regular spheroidal shape of the clusters, indicating a uniform centrifugal growth; 3) similar clusters are found nowhere else, either in the yolk-sac or the body proper; if these clusters have been simply swept to their definitive locations along the ventral wall of the aorta by the blood current, then such clusters should be found also elsewhere, from whence they might be carried; the sinusoids of the liver, of the yolk-sac, of the heart, and the jugular veins, and the inferior vena cava, and the hepatic vein would seem to be equally favorable locations for their residence; 4) occasionally small clusters are attached to the outer sides of oblique ventral (mesonephric) vessels where these would not be expected to adhere if carried by the current and pressed against the wall; 5) if carried here by the blood, we should expect to find certain clusters elsewhere in the aorta except attached to its ventral wall; in certain portions where clusters appear on the ventral wall the aorta is packed with late erythrocytes, but no hemoblast clusters are found among them; 6) the blood stream also moves toward the dorso-lateral branches, but such never contain cell clusters; 7) the strongest current of the blood in the aorta would seem to be in the direction of the long axis of the aorta, that is caudad rather than ventrad, causing a jamming of clusters (if originally free) in the terminal portion and branches, but here clusters are entirely lacking; 8) if, as originally free clusters, they were simply carried by the current, they would be expected to lodge in the mouths of the ventral aortic branches, which is not the case; 9) the clusters show a progressive increase in size corresponding with the age of the embryos, between 5 and 10 mm., indicating an intrinsic growth.

In connection with the last point, the condition shown in a 5 mm. mongoose embryo (Helly fixation; Delafield's hematoxylin toto stain) is of the greatest importance. Here a few small clusters occur along the ventral portion of the aorta. They are generally located lateral

to the ventral mid-line, just dorsad of the mouths of the mesonephric branches; several are located just ventrad of the lateral mid-line. Transition stages can here be traced between a 'cluster' of a single cell, with hemoblast characteristics, although still attached as a spheroidal cell to the endothelial wall, and clusters of approximately a dozen cells. Certain small clusters appear as if the endothelium had buckled into the aorta. Slightly larger clusters consist of a core of endothelial cells passing into intimately associated peripheral hemoblasts. The latter condition is easily comprehensible as a derivation from the evaginated endothelium through proliferation and peripheral differentiation. In a 16 day Loggerhead turtle embryo hemoblasts can be seen differentiating from endothelium even slightly dorsad of the lateral mid-line.

There seems to be no escape from the interpretation of such clusters of hemoblasts in the pig and other embryos as derivatives from the ventral endothelium of the aorta.

The further question then arises: why is the hemogenic capacity of endothelium of the aorta limited to the ventral wall in the region of the mesonephroi? The evidence from the study of the yolk-sac and the capillaries and vessels surrounding the brain and cord, indicates that young endothelium has a general hemogenic capacity in the pig and certain other vertebrate embryos. The ventral portion of the aorta, from where numerous branches are sprouting at these early embryonic stages, probably contains a younger and less highly differentiated type of endothelium, with greater proliferative capacity, which may explain its hemogenic rôle. Emmel no doubt has the same idea in mind when he suggests a correlation between these clusters and the development and caudal shifting of the ventral aortic branches.